# OCCURRENCE, DISTRIBUTION AND ALTERNATIVE HOSTS OF CASSAVA VIRUSES IN KANO AND KATSINA STATES, NIGERIA

**BY**

**Mustapha Abdullahi UBALE, B. AGRIC. (ZARIA) 2010**
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**DEPARTMENT OF CROP PROTECTION,**
**FACULTY OF AGRICULTURE,**
**AHMADU BELLO UNIVERSITY,**
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## DECLARATION

I declare that the work in this Dissertation entitled "Occurrence, Distribution and Alternative Hosts of Cassava Virusesin Kano and Katsina States, Nigeria" was carried out by me in the Department of Crop Protection. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another Degree or Diploma at this or any other Institution.

Mustapha Ubale Abdullahi ____________________ ______________________
(Student) Signature Date

## CERTIFICATION

ThisDissertation entitled "OCCURRENCE, DISTRIBUTION AND ALTERNATIVE HOSTS OF CASSAVA VIRUSESIN KANO AND KATSINA STATES, NIGERIA" by Mustapha Abdullahi UBALE meets the regulations governing the award of the Degree of Master of Science in Crop Protection of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. O.O. Banwo __________________ ________________
(Chairman, Supervisory Committee) Signature Date

Prof. M.D. Alegbejo __________________ ________________
(Member, Supervisory Committee) Signature Date

Prof. B.D. Kashina __________________ ________________
(Member, Supervisory Committee) Signature Date

Prof. O.O. Banwo __________________ ________________
(Head, Department of Crop Protection) Signature Date

Prof. S.Z. Abubakar __________________ ________________
(Dean, School of Postgraduate Studies) Signature Date

## DEDICATION

This Dissertationis dedicated to my beloved father, late Mallam Ubale Abdullahi Angale whom I wish lived to celebrate this achievement. May you dwell in Jannatul fir daus, Ameen.

# ACKNOWLEDGEMENTS

All praise is due to Almighty Allah, who out of His infinite mercy made it possible for me to complete this work. May His peace, adoration and blessings be upon the Noblest of mankind Muhammad, his companions and those that follow their footstep till the day of reckoning.

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## ABSTRACT

Cassava mosaic virus is said to occur in all growing areas in Africa and a substantive land area is devoted to cassava in Kano and Katsina State of Nigeria. There is also the need to investigate if Cassava congo sequivirus recently reported in Kaduna and Sokoto States occurs in the States. Field surveys were conducted during the 2016 dry and wet seasons in three local government areas each of Kano and Katsina States of Nigeria to determine the occurrence, distribution and alternative hosts of viruses of cassava (*Manihot esculenta* Crantz). A total of 18 cassava farms were surveyed in the twoStates (nine in each State). Sampling was done in 5 x 5 $m^2$ quadrats in each field of the three selected fields per Local Government Area. Cassava plants with mosaic, distortion, stunting, chlorosis and necrotic symptoms on leaves, as well as, those with no obvious virus disease symptoms were sampled. A total of twenty weed samples, were also collected during both dry and wet seasons andanalysis was carried out for the presence of cassava viruses. Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) was used to detect *Cassava congo sequivirus*(CCSV) while the Triple Antibody Sandwich Enzyme Linked Immunosorbent Assay (TAS-ELISA) was used to detect *African cassava mosaic virus*(ACMV) and *East african cassava mosaic virus* (EACMV). After the analyses, three Cassava viruses *Cassava congo sequivirus, African cassava mosaic virus*and *East african cassava mosaic virus* were detected either singly (ACMV 30.4 %, EACMV 21.5 %, CCSV 17.8 %) (ACMV 28.9 %, EACMV 25.2 %, CCSV 28.1 %) or in mixed infections of two or even the three viruses (ACMV + EACMV 11.1 %, ACMV + CCSV 2.2 %, EACMV + CCSV 1.5 % and ACMV + EACMV + CCSV 1.5 %) (ACMV + EACMV 9.6 %, ACMV + CCSV 14.8 %, EACMV + CCSV 5.9 % and ACMV + EACMV + CCSV 3.0 %) in all the Local Governments Areas of

Kano and KatsinaStates respectively. This provides the first report of these viruses in the States and the second report of the occurrence of CCSV in Nigeria. None of the weeds could be confirmed as hosts of the viruses.

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# CHAPTER ONE

## 1.0 INTRODUCTION

Cassava, *Manihot esculenta* Crantz, is a perennial woody shrub with an edible root, which grows in tropical and sub-tropical areas of the world (IITA, 2009). It is widely cultivated for its tuberous root that is rich in carbohydrate. It belongs to the family *Euphorbiceae* that also includes other commercially important plants like castor bean (*Ricinus communis* L.) and rubber (*Havea bransiliensis* L.). Cassava and some 90 other species make up the genus *Manihot* and it is the only widely cultivated member of this genus. It is the third most important source of calories in the tropics, after rice and maize. More than 276 million tonnes of cassava was produced worldwide in 2016, of which Africa accounted for 56 % (FAOSTAT, 2017). Nigeria produced 57.1 million tonnes making it the world's largest producer (FAOSTAT, 2017). It provides over 500 calories daily to over 70 million people (Westby, 2008).

Cassava crop is afflicted with many insect pests and diseases including cassava spider mite, cassava mealy bug(*Phenacoccus manihoti*), green mite (*Mononychellus tanajoa*), African cassava mosaic disease, cercospora leaf spot disease, cassava bacterial blight, anthracnose, cassava brown streak diseas, and nematodes (particularly *Meloidogyne* spp.) among others (and Ortiz, 2007). African cassava mosaic disease (ACMD) is the most severe and widespread disease caused by viruses limiting production of the crop in sub-Saharan Africa. Although Africa is the largest producer of cassava, yields in Africa are very low (estimated at 8.9 tonnes/ha) as compared to other cassava producing regions (Asia and Latin America) despite the fact that, under optimal conditions cassava can produce up to 80 tonnes/ha of roots in a 12- month culture period (Nassar and Ortiz, 2007). A number of factors are responsible for

the severely reduced yields in Africa, and the most important constraints are virus diseases, particularly cassava mosaic disease (CMD), cassava brown streak disease (CBSD) as well as bacterial blight (caused by *Xanthomonas axonopodis* pv. *manihotis*). Other constraints are poor agricultural practices and various other diseases caused by bacteria, fungi and nematodes (Hillocks and Wydra, 2002), most of which are considered of minor importance. The most important single reason for this is probably the almost ubiquitous presence of cassava mosaic disease (CMD) (Dixon *et al.,* 2003; Legg *et al*., 2006). CMD is caused by a group of cassava mosaic geminiviruses (CMGs) (*Geminiviridae*: *Begomovirus*). They comprise twinned (geminate) particles, approximately 20-30nm in size, containing a bipartite, single stranded, circular DNA genome (Harrison*et al.*, 1977).

Prior to 1995, only two African species of CMDs were recognised, *Africancassava mosaic virus*(ACMV) and *East african cassavamosaic virus*, and serology-based diagnostic techniques were used to show that the two had largely non-overlapping distributions, in which ACMV occurred throughout West, Central and much of southern Africa, whilst EACMV was largely confined to coastal East Africa, Malawi, Zimbabwe and Madagascar (Swanson and Harrison, 1994). As nucleic acid-based diagnostics were introduced in the later part of the 1990s, however, much greater complexity was revealed. A novel EACMV/ACMV recombinant was described from Uganda (EACMV-UG) (Zhou *et al.*, 1997; Deng *et al.*, 1997), *South african cassava mosaic virus*(SACMV) was reported from southern Africa (Berry and Rey, 2001) and three additional EACMV species were described (Fauquet and Stanley, 2003). These included*East african cassava mosaicCameroon virus* (EACMCV), *East africa cassava mosaicMalawi virus* (EACMMV) and *East African cassava mosaic zanzibar virus* (EACMZV). Additionally, mixed ACMV+EACMV virus infections were shown to be

common, frequently giving rise to synergistic interactions and enhanced symptom severity (Harrison *et al*., 1997). ACMV and EACMV were shown to co-occur in most areas where CMD occurred (Ogbe *et al*., 1999; Legg and Thresh, 2000; Berry and Rey, 2001). Further diagnostics and characterization work seems certain to reveal the presence of an even greater diversity of CMGs and CMG interactions (Legg and Owor, 2003). In cassava fields, yield losses due to CMD can be as high as 95.0 % (Fauquet and Fargette, 1990). Cassava plants infected with CMGs express a range of symptoms which depend on the virus species/strain, environmental conditions, and the sensitivity of the cassava host.

The most typical symptoms consist of a yellow or pale green chlorotic mosaic of leaves, commonly accompanied by distortion and crumpling. Symptoms are readily distinguished from those of mineral deficiency or cassava green mite damage as the virus induced chlorosis and malformation of leaflets is asymmetrical about the midrib. Two main methods are used to manage CMD. They include the use of CMD resistant or tolerant varieties and the implementation of sanitation techniques. The latter method consists of using only cuttings from healthy plants for planting and subsequently removing any infected plants (Ntawuruhunga *et al*., 2007).

### 1.2 Justification of the Study

Cassava (*Manihot esculenta* Crantz) is an important staple food crop of nearly a billion people in the world ( Mbanzibwa *et al*., 2011). It is the third largest carbohydrate food source within the tropical regions, after rice and corn (Ceballos *et al.,* 2004). It is referred to as a food security crop (Barrett *et al.,* 2006). Africa produces more than half of the world's cassava production. Although Nigeria is reported to be the highest producer of cassava, the average yield realized per hectare (6.2t/ha) (NAERLS, FDAE and P& PCD, 2017) is still far less than

what is obtained elsewhere e. g India (37t/ha). The reason for such more than 50 % yield loss may not be unconnected to the presence of insect pests and diseases (most especially virus diseases). African cassava mosaic virus is said to occur in all the areas where cassava is grown in Africa. Most of the work on cassava viruses in Nigeria was done in Southern guinea, derived Savannah zone and rain forest zones, however,not much work has been reported in Northern guinea and Sudan savannah zones. Over the years, there has been an increase in the production and consumption of cassava in the North West zone of Nigeria. This may not be without an increase in the incidence of cassava diseases. Kano has been reported to have the lowest average yield per hectare in this zone (NAERLS and FDAE, 2014). There is scanty information on weed hosts of cassava viruses particularly in Nigeria. Information on alternative hosts is an important tool in the disease management. Preliminary survey conducted in Kano and Katsina States revealed symptoms of CMD. It is also not clear if CCSV very recently reported in Kaduna and Sokoto States (Badamasi, 2017) is present in Kano and Katsina States. Therefore, it is necessary to carry out this research in Kano and Katsina States.

## 1.3 Objectives

The objectives of this research are to:

1. Determine the occurrence and distributionof cassava virusesin Kano and Katsina States.

2. Identify the alternative hosts of the viruses.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1. The Cassava Plant

### 2.1.1 Botany of cassava

Cassava (*Manihot esculenta* Crantz) is a dicotyledonous plant, belonging to the family Euphorbiceae (Alves, 2002). It is a perennial shrub, 2 to 4 m in height and is mainly propagated from stem cuttings. Propagation by seed results in genetically diverse plants and is used for generation of new varieties (Tivana, 2012). Cassava can also be propagated by "mini-stem" for rapid propagation and by tissue culture to propagate plants free of pathogens (Rulkens, 1993).

The ideal temperature range for growth is 24 to 30 $^{o}$C (IITA, 1990) but it can tolerate temperatures ranging from 16 to 38 $^{o}$C (Tivana, 2012). Cassava can grow in the semi-arid tropics with an annual rainfall less than 600 mm, but the ideal rainfall is 1000 to 1500 mm per year (Alves, 2002). It grows well in sandy to light soils where the storage roots can develop easily (Tivana, 2012). Cassava is a monoecious species with panicle inflorescences at the reproductive branching points, female flowers (staminate) in the base and male flowers (pistillate) at the top of the inflorescence. The root cannot be used for vegetative propagation (Alves, 2002). The roots can be left in the ground for a long time before harvesting, thus giving poor farmers a useful security against famine. These characteristics make cassava the most cheaply cultivated crop as compared to other major staple crops such as rice, maize, wheat, and sugarcane, thus making cassava convenient for small-scale farmers in many tropical countries who have limited access to expensive agricultural inputs (Mabasa, 2007).

### 2.1.2 Origin of cassava

Cassava is believed to have originated in South America and was introduced into Africa in the 16th century, and later into Asia in the late 17th century by Portuguese Traders (Mabasa, 2007). Its domestication began 5000-7000 years BC in the Amazon, Brazil (Allen, 2002). Cassava was taken from Brazil to the West coast of Africa by Portuguese navigators in the $16^{th}$ century (Jones, 1959; Nweke, 1994). Today, cassava is cultivated in more than 80 countries mainly between $30^{o}$ south and $30^{o}$ north of the equator (Fauquet and Fargette, 1990).

### 2.1.3 Cassava production

The world production of cassava has reached 276 million metric tonness of which trade accounts for about 10.0 % of the total production (FAOSTAT, 2017). This is produced on total world land area of 23,462,000ha. Africa accounts for about 56 percent of the global production with 154,419,863 tonnes from 16,830,790ha. The other major cassava producing continent is Asia with approximately 32% from estimated 4.1 million ha of cassava cultivated land. Average yield of world cassava production in 2015 was estimated to be 12.0 tonnes/ha. Africa's yields are the lowest (10 tonnes/ hectare) compared to India (26tonnes/ hectare) (James and Faleye, 2015). Nigeria is the world largest producer of cassava with 57.1 million metric tonnes produced in 2016 (FAOSTAT, 2017) (Table 1) on a land area of more than 6.2million hectares, while Mozambique is ranked 10 on the list with 9.1 million metric tonnes produced from 1.0 million hectares of land. According to (NAERLS and FDAE, 2015), the trend of cassava production is on the rise in all the geo-political zones of the country except the north east (probably due to the crisis bedeviling the zone) (Table 2).

**Table 1:** World Cassava Production

| Rank | Country | Amount produced (million metric tonnes) | Area (million hectares) |
|---|---|---|---|
| 1 | Nigeria | 57.1 | 6.26 |
| 2 | Thailand | 31.2 | 1.46 |
| 3 | Brazil | 21.1 | 1.40 |
| 4 | Indonesia | 20.7 | 0.87 |
| 5 | Ghana | 17.8 | 0.94 |
| 6 | DR Congo | 14.7 | 1.80 |
| 7 | Vietnam | 11.0 | 0.58 |
| 8 | Cambodia | 10.2 | 0.39 |
| 9 | Angola | 10.0 | 0.92 |
| 10 | Mozambique | 9.1 | 1.00 |

Source: FAOSTAT(2017)

**Table 2:** Cassava production in the six geo-political zones of Nigeria(1000 tonnes)

| Zone | 2014 | 2015 |
|---|---|---|
| North East | 2360.8 | 2357.4 |
| North West | 3122.5 | 3599.9 |
| North Central | 13372.5 | 13919.8 |
| South West | 12446.5 | 13444.7 |
| South East | 11398.0 | 11977.9 |
| South South | 11645.6 | 12594.4 |

Source: NAERLS and FDAE(2014,2015)

### 2.1.4 Cultivation of cassava

Cassava is propagated by the stem and can grow indefinitely, alternating periods of vegetative growth, storage of carbohydrates in the root, and even periods of almost dormancy (Tivana, 2012). The dormancy occurs when there are severe climatic conditions, such as low temperature or prolonged water deficit (Alves, 2002). African farmers grow cassava in mono-culture or together with maize (*Zea mays* L), bananas (*Musa acuminate* Colla), groundnuts (*Arachis hypogea* L.*), rice (Oryza sativa* L.), beans (*Phaseolus vulgaris* L.) or other crops (Fauquet and Fargette, 1990). On average, cassava storage roots are ready for harvest from 12 months after planting, except for precocious varieties that can be harvested before 12 months. The time until harvest is also dependent on environmental conditions (Tivana, 2012).

### 2.1.5 Nutritional composition of cassava

The composition of cassava depends on the specific tissue (root or leaf) and on several factors, such as geographic location, variety, age of the plant, and environmental conditions. The roots and leaves, which constitute 50.0 and 6.0 % of the mature cassava plant, respectively, are the nutritionally valuable parts of cassava (Tewe and Lutaladio, 2004). Cassava leaves contain an average of 21.0 % protein, which is high among non-leguminous plants (Ravindran, 1993). Table 3 shows the percentage of the nutrients contained in cassava root in dry and fresh forms.

**Table 3:** Nutrient Composition in 100 gram of Cassava root

| Nutrient | Fresh weight (%) | Dry weight (%) |
|---|---|---|
| Dry matter | 33.30 | 100.0 |
| Starch | 28.05 | 84.23 |
| Crude protein | 2.84 | 8.53 |
| Fat | 0.18 | 0.54 |
| Crude fibre | 1.38 | 4.14 |
| Ash | 0.85 | 2.55 |
| Calcium | 1.11 | 0.33 |

Source: Odebunmi *et al*.(2007)

**2.1.6 Uses of cassava**

Nearly every person in Africa eats about 80 kilograms of cassava per year. It is estimated that 37.0 % of dietary energy comes from cassava. The Democratic Republic (DR) of Congo is the largest consumer of cassava in sub-Saharan Africa, followed by Nigeria. Apart from food, cassava is very versatile and its derivatives and starch are applicable in many types of products such as foods, confectionery, sweeteners, glues, plywood, textiles, paper, bio-degradable products, monosodium glutamate, and drugs. Cassava chips and pellets are used in animal feed and alcohol production (IITA, 2009). The use of cassava flour is gaining ground in the production of biscuits, sausage rolls, meat pies and bread (Ogbe *et al.,* 2001). It is rich in carbohydrates, calcium, vitamins B and C, and essential minerals. However, nutrient composition differs according to variety and age of the harvested crop, soil conditions,

climate and other environmental factors during cultivation (IITA, 2009). The roots of cassava are the major source of dietary starch. In DR-Congo and several East African countries, cassava leaves are also consumed as green vegetable to provide important sources of proteins, minerals, and vitamins (Achidi *et al*., 2005). Cassava bio-fortified with vitamin A was released in 2011 and 2013 in Nigeria which thus prompted the analysis of their adoption by Nigerian farmers (Chikaire *et al.,* 2016).

## 2.1 Constraints to Cassava Production

Insect pests and diseases are the most economically important constrains to the cassava production (Herren, 1994). Insect pests infesting cassava include mealy bugs (*Phenacoccus manihoti*) (Hom), cassava green mite (*Mononychellus tanajoa*) (Bondar), cassava hornworm (*Erinnyis ello*) (Linnaeus), cassava white scales (*Aonidomytilus albus*), castor thrips (*Retithrips syriacus*) (Mayet) and whitefly (*Bemisia tabaci)* (Genn)*,* (Montero, 2003). The overall devastating effects of these insect pests may be unimaginable as whitefly alone is reported to transmit at least 40 viruses of different crops worldwide (Brown and Bird, 1992).Diseases among others include cassava bacterial blight, cassava virus diseases, cassava anthracnose disease, cassava bud necrosis, and root rots (Calvert and Thresh, 2002). Economic importance of cassava diseases depends on the extent of damage a disease causes to the productive part of cassava. In sub-Saharan Africa virus diseases of cassava are the most important (Thresh *et al*., 1994; Taylor and Fauquet, 1997; Thresh *et al*., 1997).

## 2.3 Cassava Viruses

Cassava is a vegetatively propagated crop and virus diseases cause particular problems as they are carried from one crop cycle to next through stem cuttings that are used as planting material (Mabasa, 2007). In sub-Saharan Africa virus diseases of cassava are the most important (Thresh *et al*., 1994; Taylor and Fauquet, 1997; Thresh *et al*., 1997). Patil and Fauquet (2009) reported that cassava is vulnerable to at least twenty viruses but cassava mosaic and cassava brown streak viruses are the most devastating in Africa. However there is limited information on the properties, distribution, effects and economic importance of most of these viruses (Mabasa, 2007).

Sources of cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) in cassava are believed to be viruses already present in the indigenous African flora (Legg and Hillocks, 2003). Factors influencing perpetuation of the virus diseases in cassava plant include abundance of efficient insect vectors for transmission, planting of susceptible varieties and continuous use of unclean planting materials normally selected from the previous seasons (Aloyce, 2013). Mabasa (2007) reported that only two genera are of economic importance in Africa with regard to cassava, namely *Ipomovirus*: *Cassava brown streak virus* (CBSV) and *Begomovirus*: cassava mosaic geminiviruses (CMG's) of the family *Geminiviridae* (including ACMV, EACMV, SACMV and UgV).

### 2.3.1 *African cassava mosaic virus, East african cassava mosaic virus* and*South African cassava mosaic virus*

*African cassava mosaic virus*(ACMV) and*East African cassava mosaic virus*(EACMV) are distinct species of circular single-stranded DNA viruses that are whitefly-transmitted and primarily infect cassava plants. Another member of the cassava mosaic virus found distributed

in South Africa, Malawi and Madagascar transmitted by whitefly is the *South Africancassava mosaic virus*(SACMV). *The East African cassava mosaic virus-Uganda* (EACMV-UG) is another distinct member of the *Cassava mosaic virus* found distributed in the sub-Saharan Africa (Alabi *et al.,* 2011). All the viruses have similar symptoms and transmitting vector but differ in geographic distribution. The first report of cassava mosaic disease (CMD) was from East Africa in 1894 (Legg and Fauquet, 2004). Since then, epidemics have occurred throughout the African continent resulting in great economic loss and devastating famine(Legg and Fauquet, 2004).In 1971 a resistant line of cassava, the predominant host of this plant pathogenic virus, was established and used by the International Institute of Tropical Agriculture (IITA) in Nigeria. Initially following infection of a cassava geminivirus in cassava, systemic symptoms develop (Patil and Fauquet, 2009). These symptoms include chlorotic mosaic of the leaves, leaf distortion, and stunted growth (Legg and Thresh, 2000). Infection can be overcome by the plant especially when a rapid onset of symptoms occurs. A slow onset of disease development usually correlates with death of the plant (Patil and Fauquet, 2009). Though the cassava-infecting geminiviruses cause most of their economic damage in cassava, they are able to infect other plants. The host range depends on the species of virus and most are able to be transmitted and to cause disease on plants of the genera *Nicotiana* and *Datura* (Bock and Woods, 1983).

The viruses are transmitted by whiteflies in a persistent manner. Generally, whitefly requires 3 hours feeding time to acquire the virus, a latent period of 8 hours, after which it needs 10 minutes to infect the young leaves (Fauquet and Fargette, 1990). There is variation in the literature on this score, however, with other sources citing a 4-hour acquisition time and 4-hour latent period (Thurston, 1998). Symptoms appear after a 3-5 week latent period (Fargette

*et al*., 1994). Adult whiteflies can continue to infect healthy plants 48 hours after initial acquisition of the virus (Thurston, 1998). A single whitefly is sufficient to infect the host; however, successful transmission increases when multiple infected whiteflies feed on the plant (Thurston, 1998). After entering the plant through the leaves, the virus remains in the leaf cells for 8 days (Thurston, 1998). As it is a single-stranded DNA virus, it needs to enter the nucleus of the leaf cells to replicate (Fauquet and Fargette, 1990). After this initial period, the virus enters the phloem and travels to the base of the stem and out into the branches (Thurston, 1998). Travel to the branches of the plant is much slower than travel through the stem, so cuttings of branches from infected stems may be free of disease (Thurston, 1998). It has a thermal inactivation point (TIP) of 55 $^{O}$C with longevity in vitro (LIV) of 2-4 days and dilution end point (DEP) of $10^{-3}$. Virions contain 22.0 % nucleic acid; 78.0 % protein and 20 by 30 nm in size (Stanley *et al.,* 2005: Alegbejo, 2015).

Management strategies involve early planting to escape severe viruliferous whitefly population that occurs later in the season. Cassava should be planted away from and upwind of existing sources of infection and also in large compact blocks to reduce edge effect. Intercropping cassava with maize also reduces vector population and disease incidence. Use virus-free cassava cuttings for planting. Remove and destroy early infected plants if they are few (Brunt *et al.,* 1996; Alegbejo, 2015).

### 2.3.2 Cassava brown streak disease (CBSD)

Cassava brown streak disease (CBSD) was first described in the Amani district in the Tanganyika territory (now Tanzania) in 1930 (Storey, 1936). CBSD was reported to be a major threat to food security in the coastal regions of Tanzania (Legg and Raya, 1998;

Hillocks *et al*., 1996), northern Mozambique (Hillocks *et al*., 2002) and in the coastal strip of Lake Malawi. CBSD is caused by *Cassava brown streak virus*in the genus *Ipomovirus* in the family *Potyviridae* (Monger *et al*., 2001).The genome of CBSV is about 9kb composed of positive sense single stranded linear ssRNA, and a poly (A) tail at the 3´end (Mbanzibwa *et al*., 2009). Unlike members of type species of Genus *Ipomovirus* (Colinet *et al.,* 1998) CBSV genome lacks helper component proteinase but contain PI serine proteinase that strongly suppresses RNA silencing (Mbanzibwa *et al*., 2009).The first confirmation of viral cause came from Lister (1959) with sap inoculation experiment where he was able to transmit the disease by sap transmission from cassava to herbaceous hosts and back inoculation to cassava (Aloyce, 2013). Recent report shows that CSBD is more wide spread and found at areas with higher altitude than 1000 meters above sea level (masl) as previously thought(Aloyce, 2013). These areas include Uganda (Alicai *et al*., 2007), Lake Victoria zone of Tanzania and Western Kenya (Mbazibwa *et al*., 2009a; Winter *et al*., 2010; Monger *et al*., 2010), Rwanda and Burundi (Shirima *et al*., 2012) and Democratic Republic of Congo (Mulimbi *et al*., 2012; Shirima *et al*., 2012).

As it is the case with other plant viruses, the main management strategy against CBSD involves the planting of disease-free cutting. Such healthy stock needs to be maintained through rogueing of any infected individual (Hillocks and Thresh, 2000) A combination of meristem tip culture and heat treatment together with appropriate virus indexing techniques can effectively result into cassava planting material free of CBSD (Wasswa *et al*., 2011).

### 2.3.3 *Cassava congo sequivirus*

The virus belongs to the order picornavirilae under Sequiviridae family. The *Sequiviridae* is a relatively newly recognized family of viruses. Viruses belonging to the family

*Sequiviridae*infect plants and are transmitted by insects in a semi persistent manner. The family is divided into two genera: *Sequivirus* and *Waikavirus*. Most viruses belonging to the family *Sequiviridae* function as or require a helper virus in insect transmission. The viruses have isometric particles encapsidating a monopartite, positive-sense, single-stranded RNA genome. The length of genome varies, from approximately 10 kb for sequiviruses to 12 kb for waikaviruses. The natural host range of sequiviruses includes species in several plant families (ICTV, 2017). Transmission is by aphids in a semi-persistent manner. However, it is dependent on the presence of a helper virus in the genus *Waikavirus* (Thompson *et al.*, 2017).

### 2.3.4 Alternative hosts of cassava viruses

Alternative hosts of cassava viruses include crops affected by whitefly; Tomatoes (*Solanum esculentus* M), Beans (*Phaseolus vulgaris* L.), Cowpea (*Vigna unguiculata* L), Soy beans,(*Glycine max* L.), Benniseed (*Sesamum indicum* L.), Groundnut *(Arachis hypogea* L.), Okra (*Abelmoschus esculentus* L), (*Amaranthus* spp. C), Cotton (*Gossypium hirsitum* L.), cucumbers (*Cucumis sativus* L.), Sweet potatoes (*Ipomea batatas* L.) squahes, Tobacco *(Nicotiana tabacum* L.), Pepper (*Capsicum* spp.) (Alegbejo and Banwo, 2005). Shoyinka *et al.*(2001) also reported *Ricinus communis*as weed host of cassava viruses in Nigeria.

### 2.3.5 Economic importance of cassava viruses

Cassava is vulnerable to at least 20 different viruses of which the viruses causing cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are the two most economically important, causing production losses worth more than US$1 billion every year (Legg *et al*., 2006; Boykin *et al*., 2017). Annual economic losses in east and central Africa are estimated to be between 1.9 and 2.7 billion US dollars (Patil and Fauquet, 2009). Losses due

to CMD in Africa have been estimated at between 14 million tonnes, representing 47.0 % of the total production (Legg, 2008).

### 2.4 Diagnosis of cassava viruses

Virus diagnosis in cassava is important for disease prevention and control. The ultimate purpose of pathogen diagnosis is to support disease management decisions and this determines which of the available methods is to be used. The presence of the disease in a field can be detected by a simple visual observation of the virus symptoms on the leaves (Freddy *et al.,* 2015). These symptoms are variable according to whether the field is moderately or severely infected. The symptoms are however sometimes indistinct, in particular for the periods of dryness, at the time of mineral deficiency of the cassava seedlings or at the time of a serious attack of cassava green acarian or cassava cochineals (CABI, 2007). This indicates the limits of diagnoses based on disease symptoms. Such obviousness should be confirmed by more significant diagnostic tests (Freddy *et al.,* 2015).

#### 2.4.1 Serological tests

The Enzyme-Linked Immunosorbant Assay (ELISA) is a simple and sensitive method for detection and quantification of virus level in the plant (Clark and Adams, 1977). ELISA has been used for the detection of several geminiviruses (Givord *et al*., 1994). Polyclonal antibodies have been used for the detection of ACMV in cassava leaf samples by the double antibody sandwich (DAS) method of enzyme-linked immunosorbent assay (ELISA; Sequeira and Harrison, 1982) and immunosorbent electron microscopy (Roberts *et al.,* 1984). The availability of a panel of monoclonal antibodies (Thomas *et al.,* 1986) gave impetus for rapid detection and discrimination of *Cassava mosaic begomoviruses* (CMBs) using triple antibody sandwich-ELISA (Thomas *et al.,* 1986: Harrison *et al.,* 2002). Although diagnosis of CMBs

by ELISA is versatile and can be used for large-scale testing of field samples in diagnostic surveys (Ogbe *et al.,* 1997), its major limitation lies in its inability to distinguish different CMBs in mixed virus infections (Thottapilly *et al.,* 2003). In addition, similarities in the coat protein epitopes of recombinant CMBs such as EACMV-UG and their parentals makes it difficult for these viruses to be successfully differentiated by ELISA (Thottapilly *et al.,* 2003). Another major problem with this technique is the cross-reaction of the antibodies produced against one geminivirus coat protein with other geminiviruses (Freddy *et al.,* 2015). Therefore, it becomes difficult to distinguish between two closely related strains/species (Nirbhay *et al*., 2010).

### 2.4.2 Molecular tests

Polymerase chain reaction is one of the recent techniques used in the identification of viruses (Duncan and Torrance, 1992). It is a routine assay for plant virus genome which is many times more sensitive than ELISA(Ogbe *et al*., 2003; Nirbhay *et al*., 2010), and has been successful in the detection of mixed infections of ACMV and EACMV-Ug (Ogbe *et al*., 2006). A single diagnostic test or assay may provide adequate information on the identity of a virus, but a combination of methods is generally needed for unequivocal diagnosis (Acheremu, 2011). However, it is expensive technique, requiring high cost of equipment, reagents and expertise (Maniatis *et al.,* 1982).

# CHAPTER THREE

## 3.0. MATERIALS AND METHODS

### 3.1 Survey and Sampling of Cassava Fields

Surveys were conducted in January, 2016 dry season to determine the occurrence, distribution and alternative hosts of viruses of cassava in Kano and Katsina States of Nigeria. Three Local Government Areas (LGAs) each per State and three cassava farms each per LGA were surveyed for the disease incidence. The LGAs were selected based on production figures, zones and advice from members of the Cassava Growers Association of Nigeria of both States. In Katsina State, Batsari, Bakori and Dutsi Local Government Areas were surveyed while Wudil, Ungogo and Gwarzo LGAs were surveyed in Kano State. The maps of the LGAs surveyed in Kano and Katsina States are shown on Figures 1 and 2, respectively. In each farm, five quadrats 5 m by 5 m were demarcated at four corners of each field and one at the centre. The total number of plants assessed within a quadrat was recorded and the incidence of disease was calculated.

Leaf samples (15) of both symptomatic and asymptomatic cassava plants were collected from each sub-plot, properly labeled and wrapped in polyethylene bags. These were placed in an ice chest and transported to the Virology Laboratory of the Department of Crop Protection, Ahmadu Bello University (ABU), Zaria, Nigeria for diagnosis. During the survey, weed samples within and around (not more than 5 m way from the cassava farms) were sampled and randomly collected, labeled and wrapped in polyethylene bags, stored in an ice chest and transported to the Virology Laboratory of the Department of Crop Protection at ABU, Zaria. Survey for weed hosts of these viruses was repeated during the wet season in August, 2016

and the weeds were identified in the Weed Laboratory, Department of Agronomy, ABU, Zaria.

A structured survey questionnaire (Appendix I) was used to collect the following field data: Name of LGA and location of the farm, name of farmer, co-ordinates of the location of the farm (using geographical positioning system), elevation, farm size, cropping history, cropping pattern, crops surrounding farm, crop protection practices employed, field hygiene, age of crop, observed symptoms on cassava and weed samples, source of cassava stem cuttings, and varieties of cassava grown.

### 3.1.1 Disease incidence

Incidence was recorded as the population of samples with symptoms against the total number of leaf samples in study plot.Percentage incidence was calculated using the formula described by Chaube and Pundhir(2005);

$$\text{Disease incidence } (\%) = \frac{\text{Number of symptomatic samples}}{\text{Total number of samples assessed}} \times 100.$$

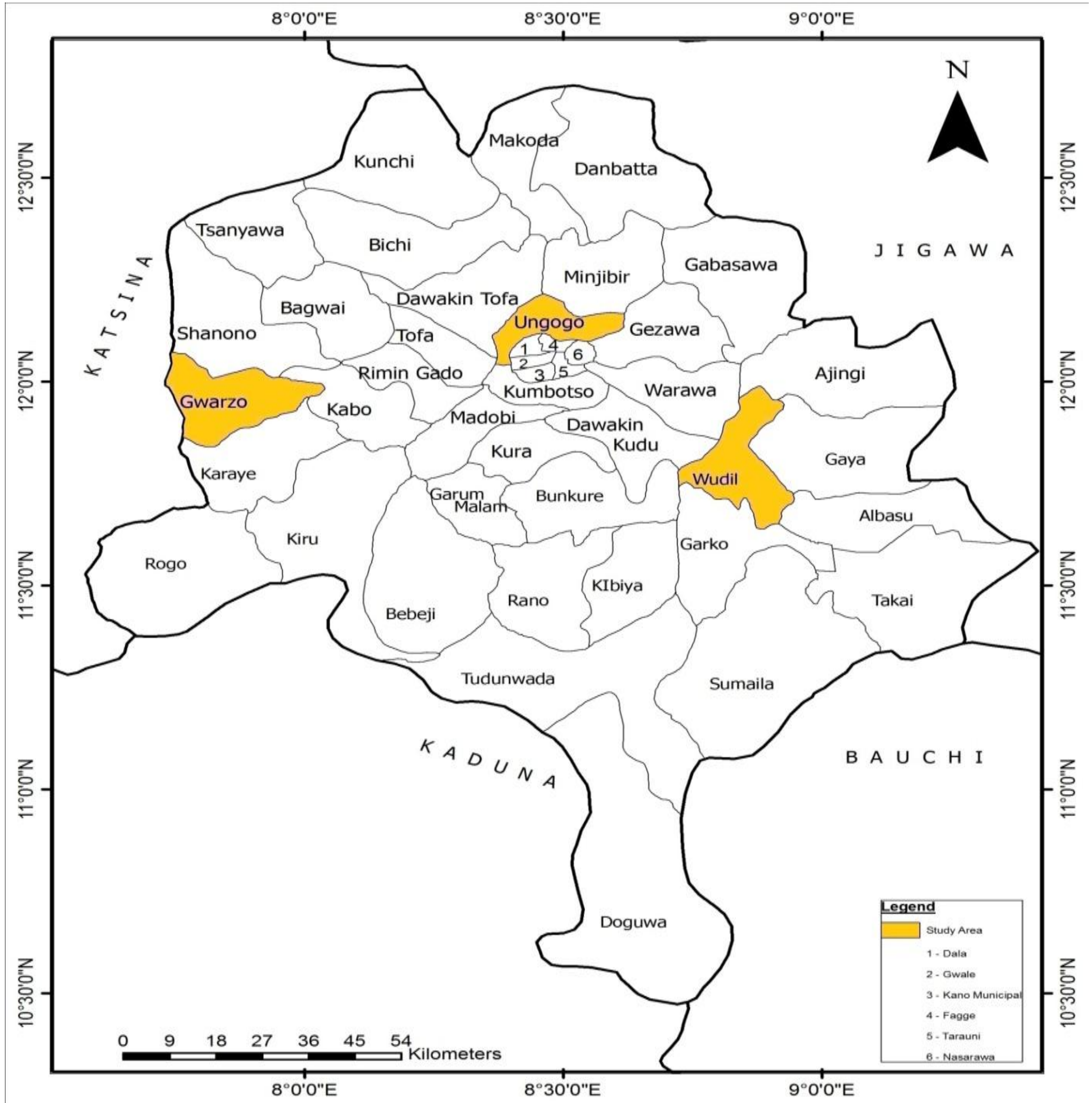


**Figure 1**: The Local Government Areas surveyed in Kano State for cassava viruses during the 2016 dry season.

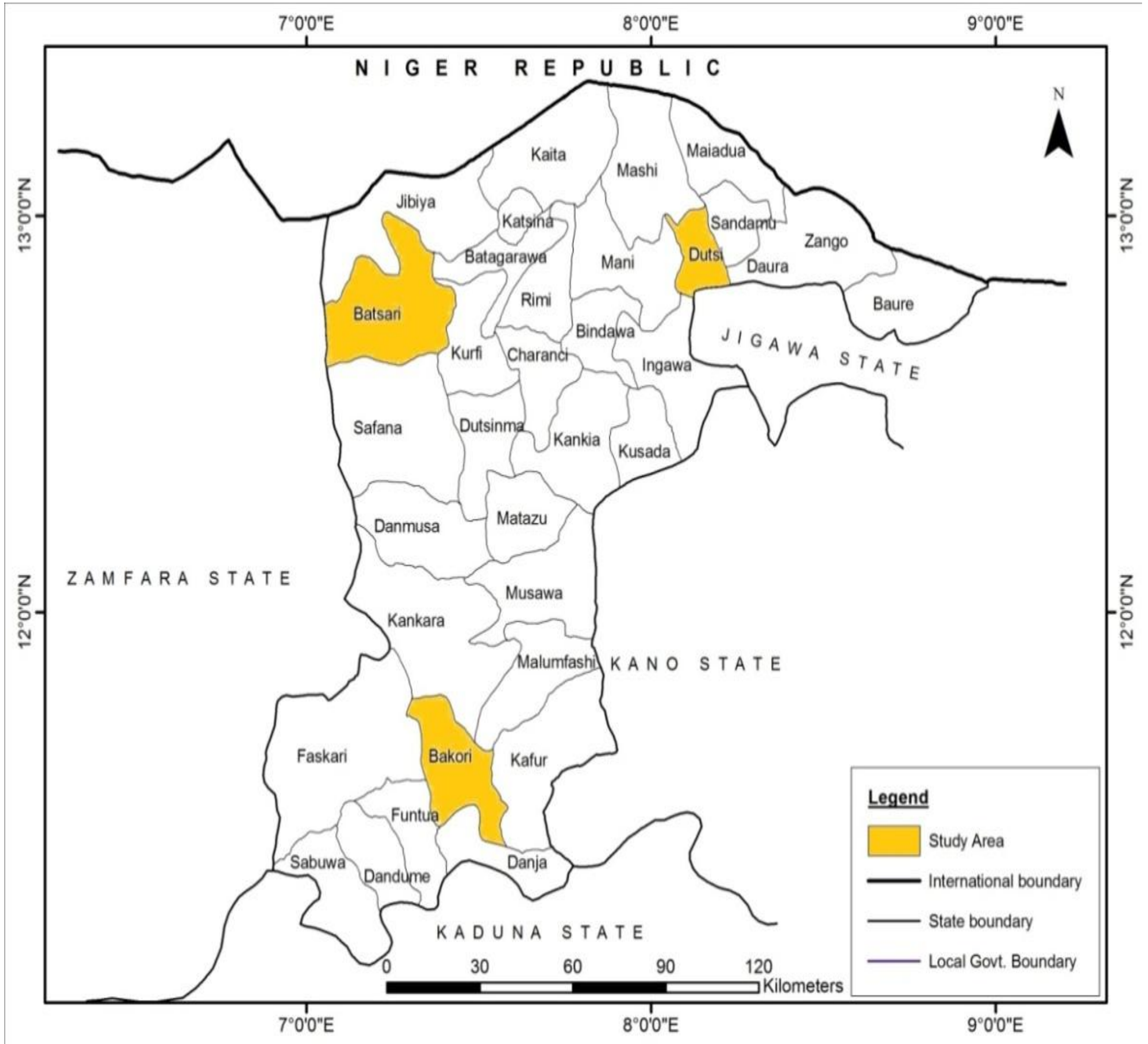


**Figure 2**: The Local Government Areas surveyed in Katsina State for cassava viruses during the 2016 dry season.

## 3.2 Detection of *Cassava Mosaic Virus* Using Enzyme-linked Immunosorbent Assay (ELISA) Techniques

### 3.2.1Triple Antibody Sandwch Enzyme-Linked Immunosorbent Assay (TAS-ELISA) For the Detection of ACMV and EACMV in Cassava leaves

The triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) was adopted for both ACMV and EACMV. Detection of the virus was done using commercially prepared polyclonal and monoclonal antibodies (DSMZ, Germany). The TAS-ELISA procedure wasas described by Clark and Adams (1977) and Givord *et al.* (1994). Polyclonal antibody (PAb) (AS-0421/4DSMZ, Germany) was diluted in coating buffer, pH 9.6 at 1:1000 µl (v/v) and 200 µl was added to each well of the microtiter plates. They were incubated at $37^0$ C for 4hour. The plates were washed with phosphate buffered saline-Tween 20 (PBS-T) after soaking for about 3 minutes. The washing was repeated two more times. The plates were blotted by tapping upside down on tissue paper. Blocking of uncoated sites was done using 200 µl of 2 % skimmed milk in PBS-T added to each well. The plates were covered and incubated for 30 minutes at $37^0$ C and tapped dry afterwards. Leaf tissue from the test samples (1 g ) was cut and put into mortars. 200 µl of extraction buffer was added to the cut leaf 1/20 (w/v) and ground to get the sap. 200 µl aliquots of the test sample were added to duplicate wells. The plates were covered and incubated overnight at $4^0$ C and washed three times as done earlier. 200 µl the monoclonal antibody (MAb) was added to each well. The plates were covered and incubated for 2 hours at $37^0$ C, and washed three times with PBS-T. Rat anti-mouse alkaline phosphatase (RAM-AP) was diluted at 1: 1000 (v/v) in conjugate buffer and 200 µl was added to each well. The plates were covered and incubated for one hour at $37^0$ C after which they were washed three times using PBS-T. Two hundred microlitre aliquots of substrate (para-nitrophenyl-phosphate in substrate buffer 1mg/ml) were added to each well

and incubated at $37^0$ C in the dark for one hour. The plates were read at $A_{405\ nm}$with an ELISA reader (Biochrom EZ Read 400). ELISA values at least twice the negative control were rated positive as reported by Kumar (2009).

The same procedure as in ACMV was employed for polyclonal antibody except for the monoclonal antibody to EACMV was used AS-0421/2.

### 3.2.2DAS-ELISA for thedetection of *cassava congo sequivirus*(CCSV) in cassava leaf samples

Detection of the virus was done using commercially prepared polyclonal and monoclonal antibodies (DSMZ, Germany). The DAS-ELISA procedure was adopted as described by Clark and Adams (1977). Polyclonal antibody (PAb) (AS-0421/4) (DSMZ, Germany) was diluted in coating buffer, pH 9.6 at 1:1000 (v/v) and 200 µl was added to each well of the microtiter plates. It was incubated at $37^0$ C for 4hours. The plates were washed with phosphate buffered saline-Tween-20 (PBS-T) after soaking for 3 minutes. The washing was repeated two more timesand blotted by tapping upside down on tissue paper. The plates were covered and incubated for 30 minutes at $37^0$ C. The plates were then removed and tapped dry. Leaf portions(1g) were cut and put into mortars. Twenty microlitres of extraction buffer was added to the cut leaf 1/20 (w/v) and ground to get the sap. Two hundred microlitres aliquots of the test sample were added to duplicate wells. The plates were covered and incubated overnight at $4^0$ C. The plates were washed three times with PBS-T. The anti-virus conjugate (IgG-AP) was diluted in conjugate buffer at 1:500 and Two hundred microlitres was added to each well. The plates were covered and incubated for 2 hours at $37^0$ C. The plates were washed three times with PBS-T. Two hundred microlitres aliquot of freshly prepared substrate (para-nitrophenyl-phosphate in substrate buffer (1mg/ml) were added to each well. The plates were covered and

incubated at $37^0$ C for 1 hour. The results were assessed using ELISA reader (Biochrom EZ Read 400) at $A_{405\ nm}$. ELISA values at least twice the negative control were rated positive as reported by Kumar (2009).

# CHAPTER FOUR

# 4.0 RESULTS

## 4.1 Symptoms of Cassava Viruses in the Fields

The different symptoms observed on the farms include mosaic, leaf distortion, chlorosis, and blisters on leaf. Others were stunted growth of the whole plant, leaf defoliation and various combination of the symptoms. The symptomof the African cassava mosaic was the most conspicuous and known symptom. For the *Cassava congo sequivirus*, any alteration from the normal known plant was considered abnormal. The aforementioned symptoms are shown on Plate I. Samples that tested positive for ACMV, EACMV and CCSV had one or more of the symptoms. Viruses occurred both in single and mixed infection of the two or three viruses.

## 4.2 Incidence of the three Cassava Viruses in Katsina State

The ELISA results showed that out of forty five samples tested from Dutsi LGA, thirty eight were positive. *Cassava congo sequivirus* (CCSV) had the highest incidence of 33.3 %, followed by *East africancassava mosaic virus*(EACMV) with 26.7 % and the least (24.4 %) was recorded for*African cassava mosaic virus*(ACMV). The ACMV + EACMV had incidence of 11.1 %, ACMV + CCSV (17.8 %), EACMV + CCSV (8.9 %), while ACMV + EACMV + CCSV had incidence of 6.7% as shown in Figure 3.

Thirty three samples tested positive from forty five in Bakori Local Government Area. EACMV had the highest incidence of 26.7 % followed by ACMV with incidence of 24.4 % and the least incidence of 22.2 % was observed for CCSV. ACMV + EACMV had incidence of 4.4 %, ACMV + CCSV 13.3 %, EACMV + CCSV had incidence of 2.2 %, while no incidence of ACMV + EACMV + CCSV was detected in the samples (Figure 3).

Plate I: Cassava leaves showing symptoms*Cassava congo sequivirus* (CCSV) (B) and *African cassava mosaic virus* (ACMV) (C) compared with healthy leaf(A).

In Batsari Local Government Area, forty out of the forty five samples tested positive. ACMV had the highest incidence of 37.8 % followed by CCSV with 28.9 % and the least was EACMV with 22.2 %. ACMV + EACMV, ACMV + CCSV had the same incidence of 13.3 %, EACMV + CCSV had 6.7 % incidence while ACMV + EACMV + CCSV had 2.2 % incidence as shown in Figure 3.

### 4.3 Incidences of the three cassava Viruses in Kano State

The ELISA results for the cassava leaf samples collected in Wudil Local Government Area showed that out of forty five samples tested, twenty were positive. The highest incidence was recorded for ACMV 28.8 % followed by EACMV with incidence of 11.1 % and the least was 4.4 % for CCSV. As for mixed infections, ACMV + EACMV had 8.9 % incidence, while ACMV + CCSV, EACMV + CCSV and ACMV + EACMV + CCSV all had the same incidence of 2.2 % as shown in Figure 4.

Twenty two out of the forty five samples tested in Ungogo Local Government were positive. ACMV + EACMV had the same incidence of 20.0 % which was higher than that of CCSV (8.9 %). For the mixed infections, ACMV + EACMV had 8.9 % incidence while ACMV + CCSV + EACMV and CCSV + ACMV and EACMV + CCSV all had no incidence in the samples (Figure 4).

In Gwarzo Local Government Area, forty three out of forty five samples tested were positive. ACMV had the highest incidence of 40.0 % followed by EACMV with 33.3 % incidence and the least was recorded by CCSV (22.2 %). For the mixed infections, ACMV + EACMV had incidence of 15.6 %, ACMV + CCSV had 4.4 % incidence while EACMV + CCSV + ACMVand EACMV + CCSV both had the same incidence of 2.2 % (Figure 4)

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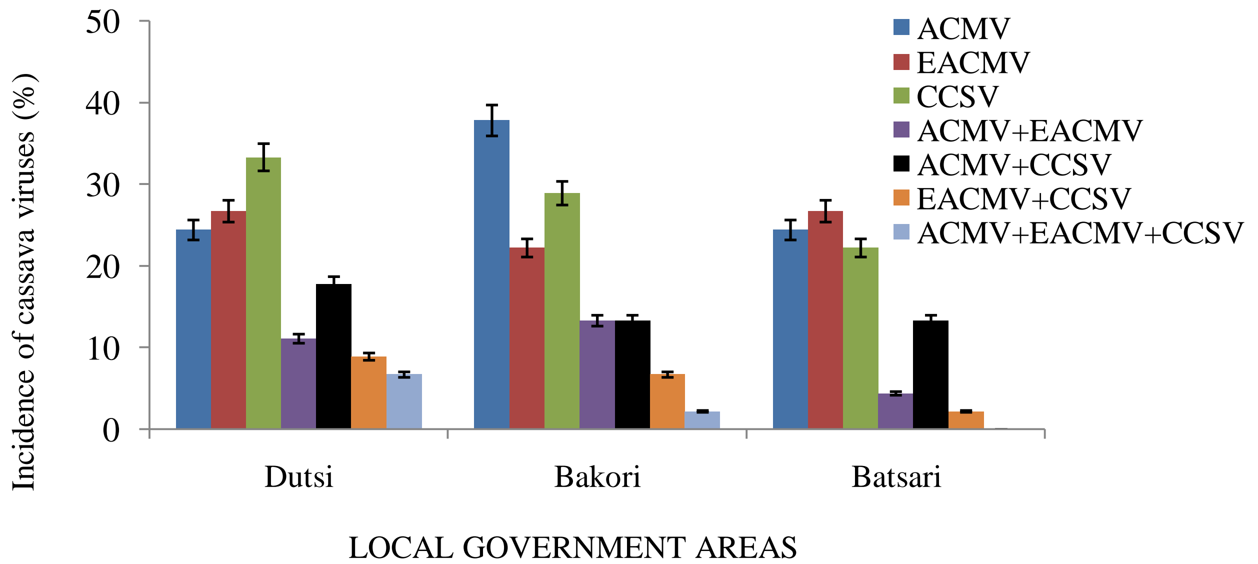


**Figure 3**: Incidence of cassava viruses in Katsina State, during the 2016 dry season.

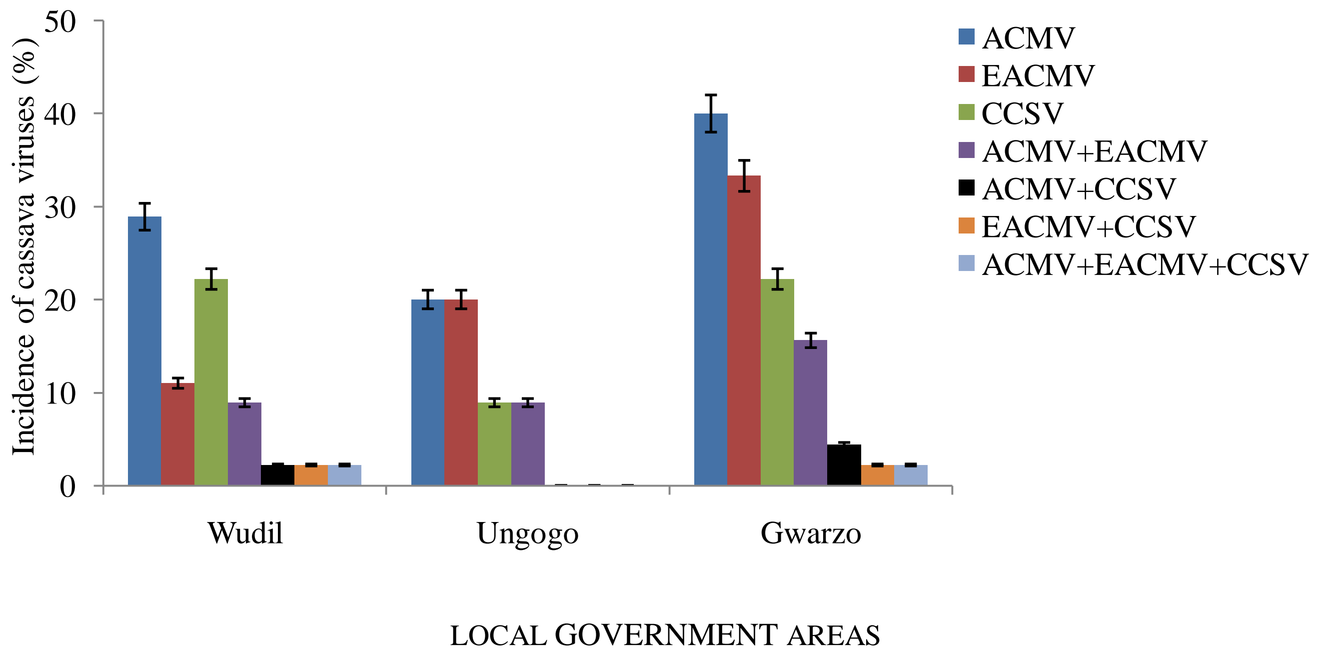


**Figure 4**: Incidence of cassava viruses in Kano State, during the 2016 dryseason

### 4.4 Distribution of Viruses Infecting Cassava in Katsina and Kano States.

Viruses of cassava detected in Katsina and Kano States were found to occur both singly and in mixture. In Katsina State, ACMV had the highest occurrence of 25 % followed by CCSV with 24 % and EACMV had 22 %. ACMV + CCSV had 13 % while ACMV + EACMV had 8 %, EACMV + CCSV had 5 % and the least was ACMV + EACMV + CCSV with 3 % as shown in Figure 5A.

In Kano State, ACMV had the highest percentage occurrence as in Katsina but with 35 % followed by EACMV with 25 % and CCSV had the occurrence of 21 %. ACMV + EACMV had the occurrence of 13 %, ACMV + CCSV 3 % while EACMV + CCSV and ACMV + EACMV + CCSV jointly had the least occurrence of 2 % in the State as depicted in Figure 5B.

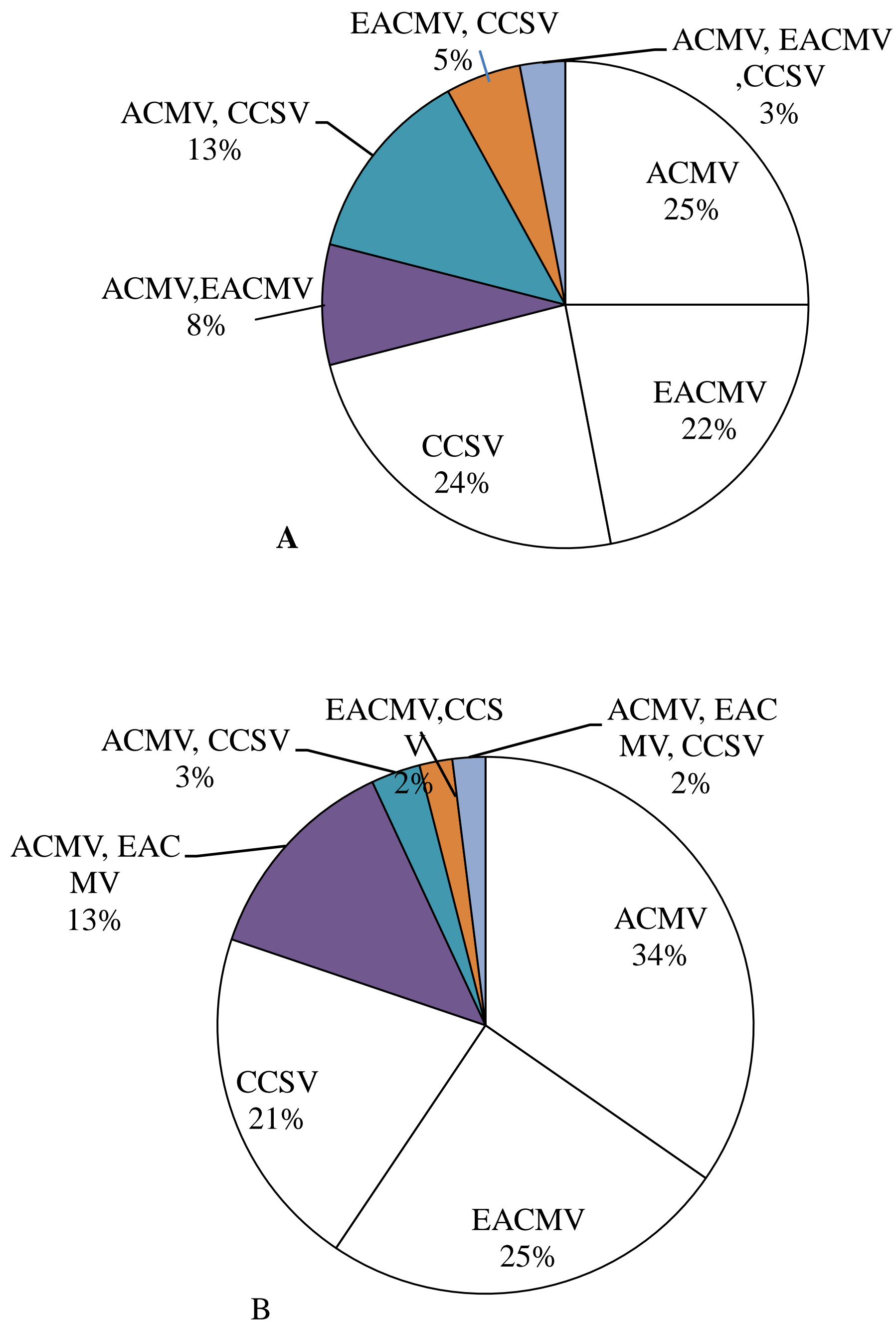


**Figure 5**: Distribution of Viruses Infecting Cassava in (**A**) Katsina and (**B**) Kano States during the 2016 dry season.

## 4.5 Distribution of Viruses Infecting Cassava in Kano and Katsina States.

The distribution of the viruses infecting Cassava in Kano State (Table 4) varies considerably from one Local Government Area to another. Only one location in Wudil (Tsibiri II) had all the three viruses. The remaining eight locations had at least one of the viruses detected in them. ACMV and EACMV only were detected in Koye and Bare-bari of Gwarzo and Ungogo LGAs respectively. EACMV and CCSV only were detected in Nassarawa (Gwarzo LGA) while ACMV and CCSV were detected in Madigawa and Michika of Gwarzo and Ungogo LGA respectively.

In Katsina State (Table 5), the distribution did not vary much across the nine locations. All the viruses were detected in at least one location of the three Local Government Areas. These locations included Kuya and Jar kasa (Dutsi LGA), Gidan makera (Bakori LGA), and Wagini and Makiyaya (Batsari LGA). Yalwa (Bakori LGA) had only ACMV and EACMV detected while Mahuta (Batsari LGA) had ACMV and EACMV detected. In two locations of Ganjirawa (Bakori LGA) and Dutsawa (Dutsi LGA), only EACMV was detected. The different locations in the states and the viruses detected are shown in Figures 6 and 7 in Katsina and Kano States respectively.

**Table 4:** Distribution of African Cassava Mosaic Virus, East African Cassava Mosaic Virus and Cassava Congo Sequivirus in three Local Government Areas of Kano State during the 2016 dry season.

| LGA | Location | Number of samples tested | Cassava viruses detected | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Single infection | | | Mixed infections | | | |
| | | | ACMV | EACMV | CCSV | ACMV +EACMV | ACMV +CCSV | EACMV +CCSV | ACMV +EACMV + CCSV |
| Wudil | Kayu | 15 | - | - | 6(+) | - | - | - | - |
| | Tsibiri I | 15 | - | - | 2(+) | - | - | - | - |
| | Tsibiri II | 15 | 13 (+) | 5 (+) | 2(+) | 4(+) | + | + | + |
| Ungogo | Michika | 15 | + | - | 4(+) | - | - | - | - |
| | Michika gari | 15 | - | 3(+) | - | - | - | - | - |
| | Bare-bari | 15 | 9 (+) | 6(+) | - | 4(+) | - | - | - |
| Gwarzo | Madigawa | 15 | 4 (+) | - | 6(+) | - | - | - | - |
| | Koye | 15 | 14 (+) | 8(+) | - | 7(+) | - | - | - |
| | Nassarawa | 15 | - | 7(+) | 4(+) | - | 2(+) | + | + |
| Total | | 135 | 41 | 29 | 24 | 15 | 3 | 2 | 2 |

ACMV= African Cassava Mosaic Virus, EACMV=East African Cassava Mosaic Virus, CCSV= Cassava Congo Sequivirus, += Detection, - = No detection.

Numbers before + = number of positive samples

**Table 5**; Distribution of African Cassava Mosaic Virus, East African Cassava Mosaic Virus and Cassava Congo Sequivirus in three Local Government Areas of Katsina State during the 2016 dry season.

| LGA | Location | Number of samples | Cassava viruses detected | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Single infection | | | Mixed infections | | | |
| | | | ACMV | EACMV | CCSV | ACMV +EACMV | ACMV +CCSV | EACMV + CCSV | ACMV +EACMV + CCSV |
| Dutsi | Kuya | 15 | 8(+) | 10(+) | 4(+) | 5(+) | 3(+) | 3(+) | 2(+) |
| | Dutsawa | 15 | - | + | - | - | - | - | - |
| | Jar kasa | 15 | 3(+) | + | 11(+) | - | 5(+) | + | + |
| Bakori | Gidan makera | 15 | 4(+) | 11(+) | 3(+) | 2(+) | + | + | - |
| | Ganjirawa | 15 | - | + | - | - | - | - | - |
| | Yalwa | 15 | 7(+) | - | 7(+) | - | 5(+) | - | - |
| Batsari | Wagini | 15 | 5(+) | 8(+) | 4(+) | 4(+) | 2(+) | 3(+) | - |
| | Makiyaya | 15 | 7(+) | + | 9(+) | + | 4(+) | - | - |
| | Mahuta | 15 | 5(+) | + | - | + | - | - | + |
| Total | | 135 | 39 | 34 | 38 | 13 | 20 | 8 | 4 |

ACMV= African Cassava Mosaic Virus, EACMV=East African Cassava Mosaic Virus, CCSV= Cassava Congo Sequivirus, += Detection, - = No detection.

Numbers before + = number of positive samples

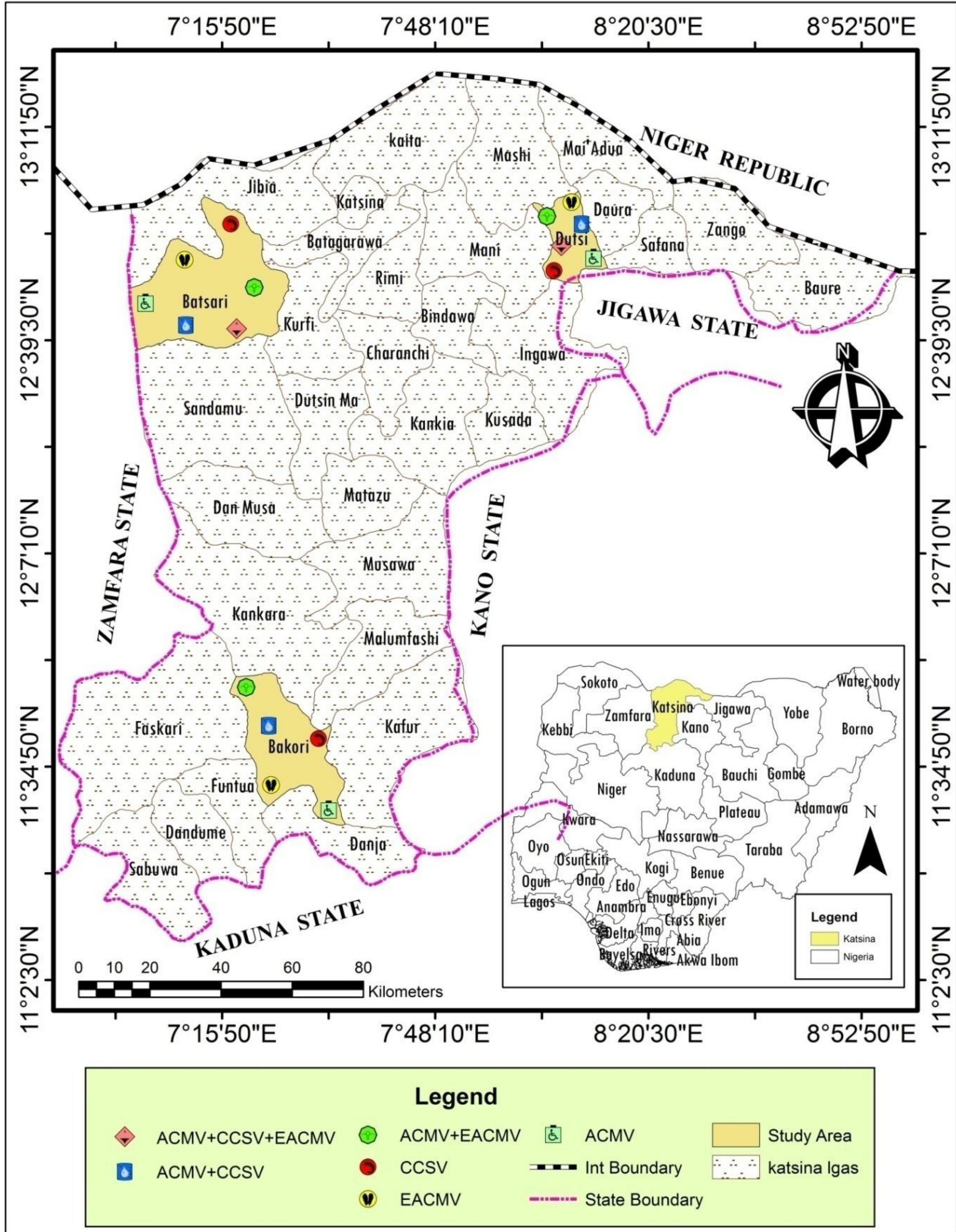


**Figure 6**: Distribution of *African cassava mosaic virus*, *East african cassava mosaicvirus* and *Cassava congo sequivirus* in Katsina State during the 2016 dry season

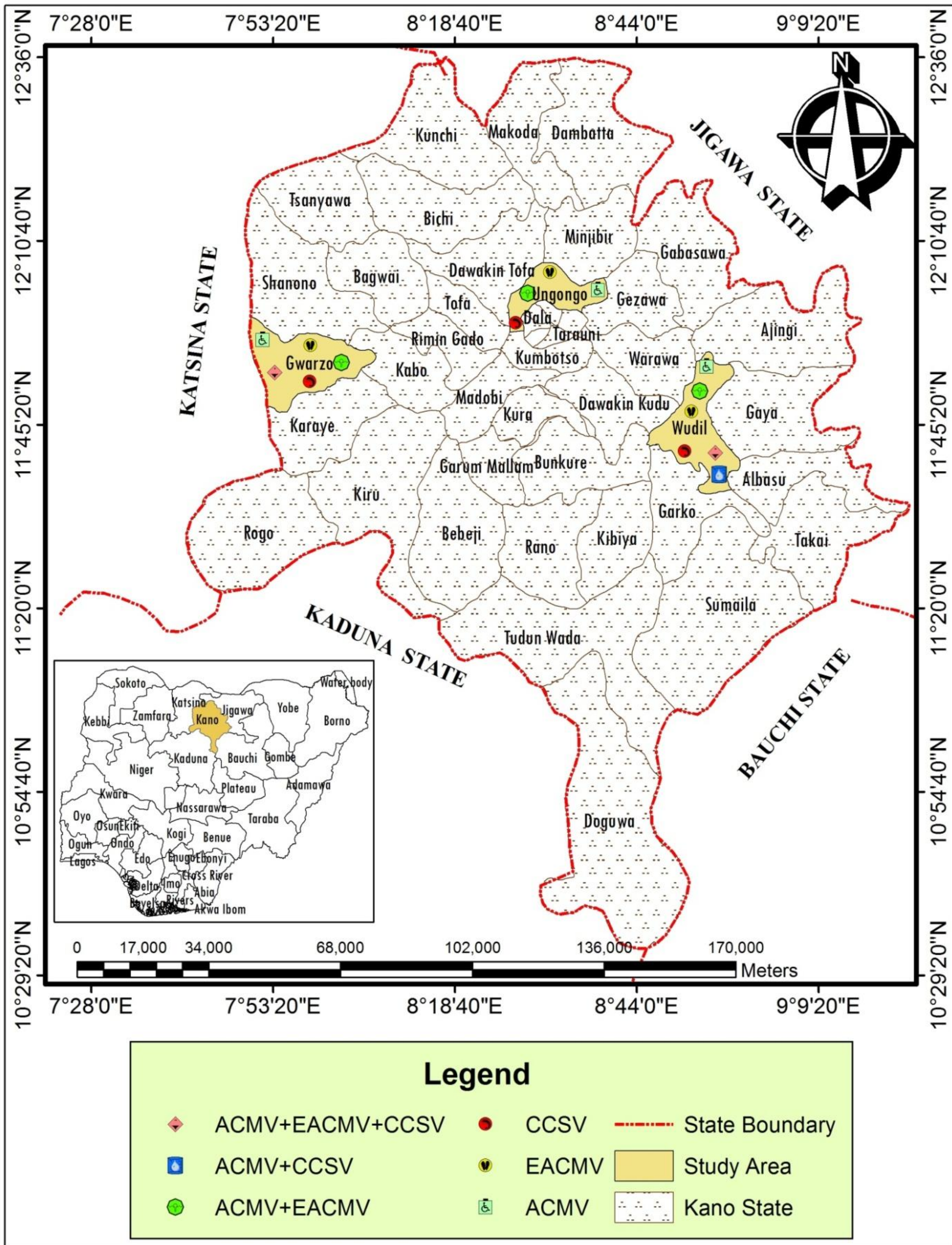


**Figure 7**: Distribution of*African cassava mosaic virus*, *East african cassava mosaic virus* and *Cassava congo sequivirus* in Kano State during the 2016 dry season

### 4.6 Alternative hosts of Cassava Viruses in Katsina and Kano States

Crop plants and samples found within and around the various locations surveyed, were also tested against the viruses. These included common weeds and other crops found growing on the cassava fields. A total of twenty nine weed samples were tested both in the dry and wet season (Table 6). The test did not confirm the host status of any of them due to small sample size and even those found positive at one season, were not available during the other season.

Table 6: Alternative hosts of cassava viruses in Katsinaand Kano States

| Plant species | ACMV | | EACMV | | CCSV | |
|---|---|---|---|---|---|---|
| | Dry season | Wet Season | Dry season | Wet Season | Dry season | Wet Season |
| *Vigna unguiculata* | + | NF | + | NF | + | NF |
| *Piliostigma thoningi* | - | - | - | - | - | - |
| *Metracarpus villosus* | - | NF | - | NF | - | NF |
| *Cassia obtusifolia* | - | NF | + | NF | - | NF |
| *Amaranthus spinosus* | - | NF | - | NF | - | NF |
| *Crotolaria retusa* | - | NF | - | NF | + | NF |
| *Tridax procumbense* | - | NF | - | NF | - | NF |
| *Schwenkia americana* | - | NF | - | NF | + | NF |
| *Gomphrena celosiodes* | - | NF | - | NF | - | NF |
| *Euphorbia heterophylla* | - | NF | - | NF | + | NF |
| *Ageratum conyzoides* | - | NF | - | NF | + | NF |
| *Cynodon dactylon* | - | NF | - | NF | - | NF |
| *Ipomea eriocarpa* | - | NF | - | NF | - | NF |
| *Arachis hypogeal* | NF | - | NF | - | NF | - |
| *Moringa oleifera* | NF | - | NF | - | NF | - |
| *Melonchia* sp | NF | - | NF | - | NF | - |
| *Solanum lycopersicum* | NF | - | NF | - | NF | - |
| *Solanum melongena* | NF | - | NF | - | NF | - |
| *Digitaria gayana* | NF | - | NF | - | NF | - |
| *Paspalum scrobiculatum* | NF | - | NF | - | NF | - |
| *Euphorbia hirta* | NF | - | NF | - | NF | - |

ACMV= African Cassava Mosaic Virus, EACMV= East African Cassava Mosaic Virus, CCSV= Cassava Congo Sequivirus, - = Not detected, + = Detected, NF= Not found.

# CHAPTER FIVE

## 5.0 DISCUSSION

The incidence, distribution and alternative hosts of three cassava viruses (*African cassava mosaic virus*, *East african cassava mosaic virus*and *Cassava congo sequivirus*) in Kano and Katsina States were established in this study. The symptoms observed on the samples which included mosaic, leaf distortion, mottling, chlorosis, stunting and reduced root size have been associated with cassava viruses (Sseruwagi *et al.,* 2004). The survey was spread to capture areas of low and high production of cassava in both States. The cropping pattern observed in both states was in conformity with Fauquet and Fargette (1990) who reported that Africans grow cassava in mono- culture and together with such crops as maize and groundnut among others. It was found that most of the cassava growers in the two States are small scale farmers with an overall average farm size of 0.61ha (Apppendix II). The average farm size for Katsina State (0.85ha) was higher than that of Kano State (0.37ha) (Appendix II). The small size nature of the farmers' fields may not convince a financial institution to offer them credit facilities necessary for obtaining improved varieties and other inputs. This could have improved the farmers' output as well as manage the viruses better.This agrees with Mabasa (2007) who reported cassava as a convenient crop for small-scale farmers due to limited access to agricultural inputs. In both States, the crop is grown at altitude between 406 m (Tsibiri) and 661 m (Gidan Makera)in Kano and Katsina, respectively. The most pronounced symptom was mosaic on leaves for which the two viruses (ACMV and EACMV) got their names.

Some of the samples reacted positively indicating the presence of the viruses. Symptom expression is influenced by several factors including: virus species/strain (Gibson, 1994;

Harrison *et al*., 1997; Owor, 2003), host response (Otim-Nape, 1993; Byabakama *et al*., 1997; Otim-Nape et al., 1998; Sserubombwe *et al*., 2001), plant age at infection (Otim-Nape, 1987; Fargette *et al*., 1988), temperature (Storey and Nichols, 1938; Chant, 1959; Kartha and Gamborg, 1975; Massala, 1987; Gibson, 1994) and soil fertility (Mollard, 1987; Spittel and VanHuis, 2000; Sseruwagi *et al*., 2003). In this study, symptoms expression could be primarily attributed to the host response with less contribution from other factors Mathias (2016).

The result of laboratory analysis confirmed the detection of the viruses (*African cassava mosaic virus*, *East african cassava mosaic virus*and *Cassava congo virus*) in Bakori, Batsari and Dutsi Local Governments of Katsina State and Ungogo, Wudil and Gwarzo Local Governments of Kano State. This could be attributed to the use of infected cuttings as planting material as most of the farmers source their cuttings locally from other farms (Appendix I) coupled with the less populations of whiteflies observed in the fields. This follows the findings of Mathias (2016) that at low whitefly population, cassava mosaic disease is primarily due to recycling of infected material. It implies that the farmers are not aware of the disease or its symptoms and thus do not put any effort into managing them. This agrees with Samura *et al.* (2014) who reported that high prevalence of *African cassava mosaic virus* is due to widespread use of local varieties which are susceptible to the disease.It implies that the most common local variety in the two States (Dan Wari) is susceptible to the three viruses. It was observed (through discussions with farmers) that farmers do not care and are mostly not aware of the occurrence of such diseases.Although Hillocks (2000) reported decrease in virus incidence with higher altitude, the higher incidence of the viruses in

Bakori(661m) than Dutsi (522m) and Batsari (505 m) LGAs, could be attributed to other reasons possibly high use of infected planting material.

Previous reports had confirmed the presence of cassava mosaic on weeds as alternate hosts (Monde *et al*., 2010; Alabi *et al*., 2008; Ogbe *et al*., 2006: Badamasi, 2017). Tomatoes (*Solanum lycopersicum* L.), Beans (*Phaseolus vulgaris* L.), Cowpea (*Vigna unguiculata* L.), Soyabeans, (*Glycine max* L.), Benniseed (*Sesamum indicum* L.), Groundnut *(Arachis hypogea* L.), Okra (*Abelmoschus esculentus* L.), (*Amaranthus* spp. C.), Cotton (*Gossypium hirsitum* L.), cucumbers (*Cucumis sativus* L.), Sweet potatoes (*Ipomea batatas* L.) squashes, Tobacco *(Nicotiana tabacum* L.), Pepper (*Capsicum* spp. L.) have been reported as alternative hosts to viruses (Alegbejo and Banwo, 2005). This study also gives a pilot report of the combination of*Cassava congo sequivirus* both singly and in mixed infections with either ACMV or EACMV or both. It further established the wider spread occurrence of EACMV as against earlier reports that it is confined to coastal regions of east Africa. It supports the report of Ogbe *et al*. (2001) that the mixed infections of ACMV + EACMV is moving from east to West Africa. ACMV was the most occurred single virus infection in all the Local Government Areas of Kano State. In Katsina State however, ACMV was most prevalent only in Bakori LGA, CCSVoccurred mostly in Dutsi while EACMV mostly occurred in Batsari LGA. EACMV had higher occurrence than CCSV in Ungogo and Gwarzo LGAs while CCSV was higher in Wudil LGA of Kano State. In Katsina State, CCSV had higher occurrence than EACMV in Dutsi and Batsari while EACMV was even the highest occurred virus amongst the three in Bakori LGA of Katsina State. The higher incidences of the viruses observed in Katsina than in Kano State could be due to more extensive cultivation (in terms of hectarage committed) of the crop in Katsina than in Kano State. The result also agrees with the report

of Aloyce (2013) that EACMV occurrence has gone far away from the coastal region deep into West Africa. Since the incidence of EACMV is increasing in Nigeria, having reported in Abia, Imo, Enugu, Anambra, Akwa Ibom, Cross river, Rivers, Delta, Ebonyi, Oyo, Ogun, Osun, Ondo (Ogbe *et al.,* 2007) and recently Sokoto and Kaduna States of Nigeria (Badamasi, 2017) and now Kano and Katsina. This confirms thereport of Carter *et al.* (1995) that the status of the virus is changing.Badamasi (2017) reported the occurrence of *Cassava congo sequivirus* in Kaduna and SokotoStates of Nigeria. This is the first report of the virus in Katsina and KanoStates. As against the report of Badamasi (2017), where CCSV only occurred in mixture with ACMV in Kaduna State only, this report had mixed infection of CCSV + ACMV, CCSV + EACMV and ACMV+ EACMV + CCSV in both Kano and Katsina States. Earlier, Dauda (2016) reported ACMV in Zaria and Igabi LGAs of Kaduna State. Also Onu (2015) reported ACMV in Kudan and Giwa LGAs of Kaduna State. Ogbe *et al.*(2006) had also reported the occurrence of ACMV + EACMV both in single and mixed infections.

Members of plant genus belonging to five families from Kano State were all negative to ACMV, EACMV, ACMV + EACMV, ACMV + CCSV AND ACMV + EACMV +CCSV during the dry season survey. Three members belonging to three families all had single infection of CCSV, however, they were not found on the field during the wet survey to confirm their alternative host nature to the virus. Eight members(*Vigna unguiculata, Piliostigma thoningi, Mitracarpus villososus, Cassia obtusifolia, Amaranthus spinosus, Ageratum conyzoides, Cynodon dactylon, Ipomea eriocarpa*)belonging to seven families were analysed from Katsina State during the dry season. Five members (*Piliostigma thoningi, Metracarpus villososus, Amaranthus spinosus,Ipomea eriocarpa, Cynodon*

*dactylon)*belonging to five families were all negative to all the viruses. One member (*Ageratum conyzoides*) belonging to Asteraceae family was only positive to CCSV. One member (*Cassia obtusifolia*) belonging to leguminaceae family was only positive to EACMV. One member belonging to Fabaceae family was positive to all the viruses in single and all combinations. However, it was not available during the wet survey in order to confirm its nature. This member (*Vigna unguiculata* L.) was specifically mentioned by Alegbejo and Banwo (2005) as alternative host to many viruses.

During the wet season survey, a total of fourteen weeds were tested for the viruses. Four members belonging to two families were from Kano State and none of them was positive to any of the viruses. From Katsina State, ten members belonging to six families were tested during the wet season. Eight of the members were negative to all the viruses. Two members belonging to two families were positive to only ACMV. Although some of the weeds were positive on the first test; none of them could be confirmed to be alternate host as they proved negative in the second test. *Cassava congo sequivirus* was detected in at least one out of the three locations in each of the local governments in the two States.

# CHAPTER SIX

## 6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

### 6.1 Summary

Field surveys were conducted on cassava farms in January 2016 to investigate the occurrence of *African cassava mosaic virus*(ACMV), *East African cassava mosaic virus*(EACMV) and *Cassava congo sequivirus*(CCSV) on cassava and also to find their possible alternative hosts in Kano and Katsina States North West Nigeria. A total of eighteen farms were surveyed, nine in each State. A total of 270 cassava leaf samples and 29 plants samples were collected during the survey from the two States. Weed survey was repeated during the rainy season to ascertain what was detected during dry season. The virus incidence and distribution were determined in each farm and then in each Local Government Area of the States. Serological method, Enzyme-Linked Immunosorbent Assay (ELISA) was employed in the detection of the viruses from the samples collected. The result revealed the presence of all the three viruses (ACMV, EACMV and CCSV) both in single and mixed infections in all the six Local Government Areas of the two States. This established a pilot report of the occurrence of particularly mixed infection of these viruses (ACMV+EACMV, ACMV+CCSV, EACMV+CCSV and ACMV+EACMV+CCSV) in the States and of CCSV. On the weeds, this study could not confirm alternative host nature of the plats tested. This was because of the limitation in running a second test to confirm the earlier result. Others were not available at either the dry or raining season survey. As such, those were found in both seasons but were only positive at one season but not in the other are only positive possibly by accident.

### 6.2 Conclusion

1. This study established the occurrence of *African cassava mosaic virus* 25 % and 34 % and*East african cassava mosaic virus* 22 % and 25 % in Katsina and Kano states, respectively.
2. It is the first report of CCSV in Katsina and Kano States (24 % and 21 %), respectively and second report in Nigeria.
3. It established the existence of mixed infections of two or three of the viruses ACMV+EACMV, EACMV+ CCSV and ACMV+ EACMV+ CCSV which was hitherto not known.
4. None of the plants tested was confirmed as alternative host to the viruses.

### 6.3 Recommendations

1. Further studies should be done on the interaction of these viruses to establish their relationship.
2. A weed host study capturing as large number of weeds as possible should be done in these States to determine their status.
3. Further search should be done to screen some locally adopted cassava varieties for resistance to the virus isolates.

**REFERENCES**

Achidi, A.U., Ajayi, O.A., Bokanga, M. and Maziya-Dixon, B. (2005). “The use of cassava leaves as food in Africa. *Ecology of Food andNutrition,* 44(66): 423-435.

Acheremu, K. (2011). Use of thermotherapy and tissue culture to free diseased cassava cultivars from cassava mosaic disease. Thesis, College of Agriculture and Natural Resources. http://hdl.handle.net/123456789/177. Accessed December 22, 2011.

Alabi, O.J., Ogbe, F.O., Bandyopadhyay, R., Kumar, P.L., Dixon, A. G. O., d’A Hughes, J. and Naidu, R. A. (2008). Alternate hosts of *African cassava mosaic virus* and *East African cassava mosaic Cameroon virus* in Nigeria. *Archives of Virology*, 153(9): 1743-1747.

Alabi, O.J. Kumar, P.L. and Naidu, R.A. (2011). Cassava mosaic disease: a curse to food security in sub-Saharan Africa. APSnet Features, 1-17.

Alegbejo, M.D. and Banwo, O.O. (2005).Host Plants of *Bemisia tabaci* Genn. in NorthernNigeria. *Journal of Plant Protection Research,* 45 (2):99-105.

Alegbejo, M.D. (2015). *Virus and virus-like diseases of crops in Nigeria*. Ahmadu Bello University Press Limited, Zaria, Nigeria. ISBN: 978-125-699-0. Pp. 168-170.

Alicai, T., Omongo, C. A., Maruthsi, M. N., Hillocks, R. J., Baguma, Y., Kawuki, R., Bua, A., Otim-Nape, G. W. and Colvin, J. (2007). Re- emergence of cassava brown streak disease in Uganda. *Plant Disease,* 91: 24–29.

Allen, A.C. (2002). The origin and taxonomy of cassava. In Hillocks, R.J., Thresh, J.M and Bellotti, A.C (Eds), *Cassava Biology, Production and Utilization.*CABI Publishing, Wallingford, UK, 1-16.

Aloyce, R.C. (2013). Development and evaluation of efficient Diagnostic tools for Cassava Mosaic and Cassava brown streak diseases. The University of Witwatersrand, Johannesburg Republic of South Africa. 1-19.

Alves, A. A. C. (2002). Cassava botany and physiology. In Hillocks, R.J., Thresh, J.M. and Bellotti A.C. (Eds). *Cassava Biology, Production and Utilization*. CABI Publishing Wallingford, UK, 67-89.

Badamasi, H. (2017). *Identification, distribution and alternative hosts of viruses of cassava in Kaduna and Sokoto States, Nigeria*. (unpublished M.Sc. dissertation). Ahmadu Bello University, Zaria. Pp 73.

Barrett, C., Morenya, P., Mc Peack, J., Minten, B., Murithi, F., Oluoch-Kosura, W., ….., and Wangika, J (2006). ‘ Welfare dynamics in rural Kenya and Mdagascar’ *Journal of Developmental Studies,* 42: 247-248.

Berry, S.D and Rey, S.E.C. (2001). Molecular evidence for diverse populations of cassava infecting begomoviruses in South Africa. *Archives of Virology.* 146: 1795-1802.

Bock, K.R..and Woods, R.D. (1983). The etiology of African cassava mosaic disease. *Plant Disease,* 67: 994–995.

Boykin, L., Locatis, M. and Brands, K. (2017). Computational Food Security. InAnonymous, Report on Flight to Denver, a solutions Summit Bootcamp, Pg. 13. Available at: http://www.projectxite.org/wp-content/uploads/2017/09/Flight-to-Denver-Solutions-Summit-Report-FINAL-Formatted.pdf. Accessed June 19, 2018.

Brown, J.K and Bird, J. (1992). Whitefly transmitted gemniviruses and associated disorders in the America and the carribean basin. *Plant Disease,* 76: 220-225.

Brunt, A.A Crabtree, K. Dallwitz, M.J. Gibbs, A.J. Watson, L and Zurcher, E.J. (eds.) (1996). Plant virus online: description and lists from the VIDE database version: URL http://biology.anu.edu.au/groups/MES/vide.

Byabakama, B.A., Adipala, E., Ogenga-Latigo, M.W., Tusiime, G., Otim- Nape, G.W., (1997). The resistance of improved cassava varieties to cassava mosaic disease in Uganda. *African Journal of Plant Protection,* 7: 45–57.

CABI. (2007). *Crop Protection Compendium*. 2007 Edition. CAB International Publishing. Wallingford, UK.

Calvert, L.A., and Thresh, J.M. (2002). The viruses and virus diseases of cassava. In Hillocks RJ, Thresh JM, Bellotti AC, editors. *Cassava: Biology, Production and Utilization*. CABI Publishing, Wallingford, UK. pp. 236–260.

Ceballos, H., Iglesias, C.A., Perez, J.C andDixon, A.G.O. (2004). Cassava breeding: opportunities and challenges *Plant Molecular Biology,*56: 503-516.

Chant, S.R. (1959). A note on the inactivation of mosaic in cassava (*Manihot utilissima* Pohl) by heat treatment. *Empire Journal of Experimental Agriculture,* 27: 55–58.

Chaube,H. S. and Pundhir, V. S. (2005). *Crop Disease and their Management*. Prentice Hall of India Private Limited. Pp 172-173.

Chikaire, J.U. Nnadi., F.N. Anaeto., F.C. Ogueri, I.E and Osuagwu, C.O. (2016). Analysis of the sociology of adoption of vitamin A cassava varieties for livestock feed among

rural farmers in Imo state, Nigeria. *African Journal of Agricultural Science and Technology,* 4(12):884-870.

Clark, M.F. and Adams, A. N.(1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal ofGeneral Virology,* 34: 475-483.

Colinet, D., Kummert, J., and Lepoivre, P. (1998). The nucleotide sequence and genome organization of the whitefly transmitted sweet potato mild mottle virus: a close relationship with members of the family Potyviridae. *Virus Resources,* 53: 187-196.

Dauda, N. (2016). *Incidence and severity of African cassava mosaic virus in Zaria and Igabi Local Government Areas of Kaduna State*. Unpublished undergraduate project. Ahmadu Bello Univeristy, Zaria. Page 34.

Deng, D., Otim-Nape, G. W., Sangare, A., Ogwal, S., Beachy, R. N., and Fauquet, C. M. (1997). Presence of a new virus closely associated with cassava mosaic outbreak in Uganda. *African Journal of Rootand Tuber Crops*, 2:23–28.

Dixon, A.G.O., Bandyoapadhyay, R., Coyne, D., Ferguson., M., Ferris, R.S.B., Hanna.,……. and Ortiz, R. (2003). Cassava: from poor farmer's crop to pace setter of African rural development. *Chronica Horticulture,* 43: 8-15.

Duncan J. and Torrance L. (1992). Techniques for the rapid detection of plant pathogens. Blackwell scientific publications.

FAOSTAT (2017).http://www.fao.org/faostat/en/#data/QC. Food and Agriculture Organisation Statistics.Accessed March 19, 2018.

Fauquet, C. M. and Fargette, D. (1990). *African cassava mosaic virus*: etiology, epidemiology and control. *Plant Disease,* 74: 404-411.

Fauquet, C.M and Stanley, J. (2003). Geminivirus classification and nomenclature: progress and problems. *Annals of Applied Biology,*142:165-189.

Fargette, D., Jeger, M., Fauquet, C. and Fishpool, L.D. (1994). Analysis of Temporal Disease Progress of African Cassava Mosaic Virus. *Phytopathology*, 54; 1 91–98.

Fargette, D., Fauquet, C. and Thouvenel, J. C.(1988). Yield losses induced by African cassava mosaic virus in relation to the mode and date of infection. *Tropical Pest Management,* 34: 89–91.

Freddy, O.B., Ndofunsu, A.D., Nakweti, R.K., Ntumbula, A.M., Hity, M., Kasali, L., …… andPhillipe, L. (2015). PCR and ELISA detection of cassava mosaic virus in a

Congolese Cassava Landrace. *International Journal of Biotechnology and Food Science,* 3 (1): 10-16.

Gibson, R.W. (1994). Long-term absence of symptoms in heat-treated African cassava mosaic geminivirus-infected resistant cassava plants. *Tropical Science,* 34: 154–158.

Givord L, Fargeite D, Kounounguissa, B. Thowenel J.C. and Van Regenmortel, M.H.V. (1994).Diagnostic d'infection à gemlnivirus à l'ade d'anticorps monoclonaux dirigés contre le virus de la mosaïque africaine du manioc. *Bulletin des Recherches Agronomiques des* Gembloux 29(2):161-168.

Harrison, B. D., Barker, H., Guthrie, E. J., Meredith, G. andAtkinson, M.(1977). Plant viruses with circular single-stranded DNA. *Nature,*270: 760-762.

Harrison, B.D., Zhou, X., Otim-Nape, G.W., Liu, Y. and Robinson, D.J. (1997). Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. *Annals of Applied Biology,* 131: 437–448.

Harrison, B. D., Swanson, M.M.and Fargette, D. (2002). Begomovirus coat protein: serology, variations and functions. *Physiology of Molecular Plant Pathology*, 60: 257-271.

Herren, H.R. (1994). Cassava Pest and Disease Management: An Overview. *African Crop ScienceJournal,* 2: 345-353.

Hillocks, R. J. (2000). Final Technical Report Management of Cassava Virus Diseases in Southern Tanzania. Https://assets.publishing.service.gov.uk/media/.../r6765ftr.pdf.

Hillocks R. J and Wydra, K. (2002). Bacterial, fungal and nematode diseases. In Hillocks R. J., Thresh, J.M and Bellotti, A.C (eds) *Cassava: Biology, Production and Utilization*, CABI, pp. 261-280.

.

Hillocks, R.J., Raya, M.D.and Thresh, J.M. (1996). The association between root necrosis and above-ground symptoms of brown streak virus infection of cassava in southern Tanzania. *International Journal of Pest Management,* 42: 285-289.

Hillocks, R.J., Thresh, J.M., Tomas, J., Botao, M., Macia, R.and Zavier, R. (2002). Cassava brown streak disease in Northern Mozambique. *International Journal of Pest Management,* 48: 179-182.

ICTV 10th Report (2017). ICTV *Virus Taxonomy*. The online 10th Report of the International Virus Committee on Taxonomy of Viruses.

IITA (1990). *Cassava in Tropical Africa*, *A reference Manual*. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 176pp.

IITA(2009). Cassava crop. International Institute of Tropical Agriculture. www.iita.org/crop/cassava.

James, D.and Faleye, T. (2015). Cassava mechanization prospects and future in Nigeria. *International Research Journal of Agricultural Science and Soil Science,*5(3): 98-102.

Jones, W.O. (1959). *Manioc in Africa*. Food research institute, Stanford, U.S.A.

Kartha, K.K. and Gamborg, O.L.(1975). Elimination of cassava mosaic disease by meristem culture. *Phytopathology,* 65: 826–828.

Klinkenberg, F.A. and Stanley, J. 1990. Encapsidation and spread of African cassava mosaic virus DNA A in the absence of DNA B when agro-inoculated to Nicotiana benthamiana. *Journal of General Virology,* 71: 1409-1412.

Kumar, P. L. (2009). *Methods of the diagnosis of Plant Viruses.A laboratory manual,* IITA, pp 62.

Legg, J.P. and Raya, M.D. (1998). Survey of virus diseases in Tanzania. *International Journal of Pest Management,* 44: 17-23.

Legg, J.and Thresh, J.M. (2000). Cassava mosaic virus disease in East Africa: a dynamic disease in a changing environment. *Virus Research*, 71: 135–149.

Legg JP, Owor B (2003). Cassava mosaic disease in Africa: where are the epidemics? African Crop Science Conference Proceedings 6:322-328.

Legg, J.P.and Thresh, J.M. (2003). Cassava virus diseases in Africa. In: *Proceedings of the First International Conference on Plant Virology in Sub-Saharan Africa* (4–8 June 2001, Ibadan, Nigeria), IITA, Ibadan, Nigeria, pp. 517–522.

Legg, J.and Fauquet, C. (2004). Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology*, 56: 585–599.

Legg, J.P., Owor, B., Sseruwagi, P and Ndunguru, J (2006). Cassava mosaic virus disease in East and Central Africa: Epidemiology and management of regional pandemic. *Advancesin Virus Research,*67: 355-418.

Legg, J.P. (2008). African Cassava Mosaic Disease. In: Elsevier (3rd Edition). *Encyclopedia of Virology*. Pp 30-36.

Lister, R.M. (1959). Mechanical transmission of cassava brown streak virus. *Nature,*183: 1588-1589.

Mabasa, K.G. (2007). *Epidemiology of cassava mosaic disease and molecular characterization of cassava mosaic viruses and their associated whitefly (Bemisia tabaci) vector in South Africa*. MSc dissertation, University of the Witwatersrand, Johannesburg.

Maniatis, T., Fritsch, E.F and Sambrook, J. (1982). *Molecular cloning*: A laboratory manual, New York. Cold spring Harbor laboratory.

Massala, R. (1987). African cassava mosaic in the Congo, its importance, distribution and methods of control. In *Proceedings of the International Seminar on African Cassava Mosaic Disease and its Control Yamoussoukro, Cˆote d'Ivoire*, 4–8 May 1987. CTA/FAO/ORSTOM/ IITA/IAPC, pp. 14 19.

Mathias, T. (2016). *Epidemiology and molecular characterization of cassava mosaic gemininviruses and their effect on yield of cassava in Zambia.* Masters dissertation, The University of Zambia, Lusaka. P. 83.

Mollard, E.(1987). African cassava mosaic among farmers of the lower Ivory Coast. In*Proceedings of the International Seminar on African Cassava Mosaic Disease and its Control, Yamoussoukro, Cˆote d'Ivoire*, 4–8 May 1987. CTA/FAO/ORSTOM/IITA/IAPC, pp. 150– 160.

Monde, G., Walangululu, J., Winter, S and Bragard, C. (2010). Dual infection by cassava begomoviruses in two leguminous species (Fabaceae) in Yangambi, Northeastern Democratic Republic of Congo. *Archives of Virology,* 155(11):1865-1869.

Mbanzibwa, D. R., Tian, Y., Mukasa, S. B. and Valkonen, J. P. T. (2009). Cassava brown streak virus (*Potyviridae*) encodes a putative Maf/ HAM1 pyrophosphatase 92.

Mbanzibwa D.R., Tian Y.P. and Tugume A. K. (2011). Simultaneous virus-specific detection of the two cassava brown streak-associated viruses by RT-PCR reveals wide distribution in East Africa, mixed infections, and infections in *Manihot glaziovii*. *Journal of Virological Methods,*171: 394–400.

Monger, W. A., Seal, S., Cotton, S. and Foster, G. D. (2001). Identification of different isolates of Cassava brown streak virus and development of a diagnostic test. *Plant Pathology,* 50: 768–775.

Monger, W.A., Alicai, T., Ndunguru, J., Kinyua, Z.M., Potts, M., Reeder, R. H…..and Smith, J. (2010). The complete genome of cassava brown streak virus and comparison with the Ugandan strain sequence. *Archives of Virology,*155: 429-433.

Montero, W.R. (2003). Cassava; Biology, Production and Utilization. *Crop Science,* 43: 448.

Mulimbi W., Phemba X., Assumani B., Kasereka P., Muyisa S., Ugentho H…..and Thom F.E. F. (2012). First report of Ugandan cassava brown streak virus on cassava in Democratic Republic of Congo. *New Disease Reports,* 26: 11.

NAERLS and FDAE. (2014). Agricultural Performance Survey Report of 2014 Wet Season in Nigeria, NAERLS Press, ABU, Zaria. ISBN: 2408-7459. Pp 142.

NAERLS and FDAE. (2015). Agricultural Performance Survey Report of 2015 Wet Season in Nigeria, NAERLS Press, ABU, Zaria. ISBN: 2408-7459. Pp 112.

NAERLS, FDAE and P & PCD. (2017). Agricultural Performance Survey Report of 2017 Wet Season in Nigeria, NAERLS Press, ABU, Zaria. ISBN: 2408-7459. Pp 129.

Nassar, N.M. A and Ortiz, R. (2007). Cassava improvement: challenges and impacts *Journal of Agricultural Science,* 145: 163-171.

Nirbhay, K., Achuit, K.S., Brotati, C. and Supriya, C. (2010). Recent advances in geminivirus detection and future perspectives. *The Journal of Plant Protection Science,* 2(1):1-18.

Ntawuruhunga, P., Okao-Okuja, G., Bembe, A., Obanbi, M., Armand Mvila, J.C. and Legg, J.P. (2007). Incidence and severity of cassava mosaic disease in the Republic of Congo. *African Crop Science Journal,*15: 1-19.

Nweke, F.I. (1994). Farm level practices relevant to cassava protection. *African Crop Science Journal,*2: 563-582.

Odebunmi, E.O.,Oluwaniyi, O.O., Sanda, A.M. and Kolade, B.O. (2007). Nutritional compositions of selected tubers and root crops used in Nigerian food preparations. *International Journal of Chemistry,*17(1): 37-43.

Oerke, E.C. (2006). Crop losses to pests. *Journal of Agricultural Science,*Volume 44/issue 01/pp 31-43.

Ogbe, F. O., Legg, J., Raya, M. D. Muimba-kankolongo, A. Theu, M. P. Kaitisha, G….and Chalwe, A. (1997). Diagnostic survey of cassava mosaic viruses in Tanzania, Malawi and Zambia. *Roots,* 4:12-15.

Ogbe, F.O., Atiri, G.I., Robinson, D., Winter, S., Dixon, A. G. O., Quin, F. M.and Thottappilly, G. (1999). First report of East African cassava mosaic begomovirus in Nigeria. *Plant Disease,* 83:398.

Ogbe F.O., Thottappilly, G., Dixon, A. G. O., Mignouna,, H.D.and Quin, F.M. (2001). Evidence of double infection and random occurrence of cassava begomoviruses in

sub-Saharan Africa. In: Roots Crops in the 21st Century. Akoroda MO, Ngeve JM (eds.), *Proc. Seventh Triennial Symp. Intern/Soc. Tropical Roots Crop African Branch (ISTRC-AB),* Cotonou, Benin Republic, pp. 524-529.

Ogbe, F.O., Atiri, G.I., Dixon, A.G.O. and Thottappilly, G. (2003).Serological and biological variations of African cassava mosaic virus in Nigeria. *Annals of Applied Biology*, 143: 203-213.

Ogbe, F.O., Dixon, A.G.O and Hughes, J. (2007). Towards the control of a severe form of cassava mosaic disease in nigeria: diagnostic survey for cassava mosaic begomovirus. Proceedings of the 13$^{th}$ ISTRC symposium, Arusha, Tanzania. Pp. 298-306.

Ogbe, F.O., Dixon, A.G.O., d'A Hughes, J., Alabi, O.J. and Okechukwu, R. (2006). Status of cassava begomoviruses and their new natural hosts in Nigeria. *Plant Diseases,* 90: 548-553.

Onu, E.G. (2015). *Incidence and severity of African cassava mosaic virus in Giwa and Kudan Local Government Areas of Kaduna State*. Unpublished undergraduate project. Ahmadu Bello Univeristy, Zaria. Page 29.

Otim-Nape, G.W.(1987). Importance, production and utilization of cassava in Uganda. In: *Proceedings of the International Seminar on African Cassava Mosaic Disease and its Control, Yamoussoukro, Cˆote d'Ivoire*, 4–8 May, 1987. CTA/FAO/ORSTOM/IITA /IAPC, pp. 203–218.

Otim-Nape, G.W. (1993). *Epidemiology of the African cassava mosaic geminivirus disease (CMD) in Uganda*. Ph.D. Thesis, University of Reading, UK, pp. 252.

Otim-Nape, G.W. Thresh, J.M., Bua, A., Baguma, Y. and Shaw, M.W. (1998). Temporal spread of cassava mosaic virus disease in a range of cassava cultivars in different agro-ecological regions of Uganda. *Annals of Applied Biology,* 133: 415–430.

Owor, B. (2003). *Effect of cassava mosaic geminiviruses (CMGs) on growth and yield of cassava mosaic disease (CMD) susceptible cultivar in Uganda and cross protection studies*. M.Sc. thesis, Makerere University, Kampala, Uganda, 134 pp.

Patil, B. and Fauquet, C. (2009). Cassava mosaic geminiviruses: actual knowledge and perspectives. *Molecular Plant Pathology*. 10: 685–701.

Ravindran, V. (1993). Cassava leaves as animal feed: Potential and limitation. *Journal of Science of Food and Agriculture,* 61: 141-150.

Roberts, I. M., Robinson, D. J. and Harrison, B. D. (1984). Serological relationships and genome homologies among geminiviruses. *Journal of General Virology,* 65:1723-1730.

Rulkens, T. (1993). Cultura de Mandioca. Apontamento de Producao Vegetal ii. Universidade Eduardo Mondlane, Maputo, Mocambique (unpublished).

Samura, A.E., Massaquoi, F.B., Mansaray, A., Kumar, P. L., Koroma, J.P., Fomba, S.N. and Dixon, A.G.O. (2014). Status and diversity of cassava mosaic disease in Serra Leon. *International Journal of Agriculture and Forestry,* 4 (3): 246-254.

Sequeira, J. C. and Harrison, B. D. (1982). Serological studies on cassava latent virus. *Annals of Applied Biology,* 101:33-42.

Shirima, R., Bigirimana, S., Gashaka, G.,Tata-Hangy, W., Barumbanze, P., Ndayihanzamaso, P….. andLegg, J.P. (2012). Cassava Brown Streak Virus rapid assessment: Burundi, the Democratic Republic of Congo and Rwanda.In *The Book of Abstracts,* International Scientific Conference of the Global Cassava Partnership for the 21st Century (GCP-21-II) June 18 – 22, 2012, the Speke Resort Conference Centre Kampala, Uganda.

Shoyinka, S. A., Thottappilly, G., McGrath, F. F. and Harrison, B. D. (2001). Detection, relationships and properties of cassava mosaic geminivirus in naturally infected castor oil plant, *Ricinus communis* L. in Nigeria. Page 20 In*The Book of Abstracts*, Fifth Conference of Cassava Biotechnology Network (CBN-V), Donald Danforth Plant Science Center, St. Louis.

Spittel, M.C and Van Huis, A. (2000). Effect of cassava mosaic disease, soil fertility, plant spacing and their interactions on cassava yields in Zanzibar. *International Journal of Pest Management* 46: 187–193.

Sserubombwe, W.S. Thresh, J.M. Otim-Nape, G.W. and Osiru, D.O.S. (2001). Progress of cassava mosaic virus disease and whitefly vector populations in single and mixed stands of four cassava varieties grown under epidemic conditions in Uganda. *Annals of Applied Biology* 135: 161–170.

Sseruwagi, P., Otim-Nape, G.W., Osiru, D.S.O.and Thresh, J.M. (2003). Influence of NPK fertiliser on populations of the whitefly vector and incidence of cassava mosaic virus disease. *African Crop Science Journal* 11: 171–179.

Sseruwagi, P., Sserumbombwe, W.S., Legg, J.P., Ndunguru, J. and Thresh, J.M. (2004). Methods for surveying the incidence and severity of cassava mosaic disease and

whitefly vector population on cassava in Africa: A review. *Virus research* 100: 129-142.

Stanley, J., Bisaro, D. M., Briddon, R. W., Brown, J. K., Fauquet, C. M., Harrison, B. D…. and Stenger, D. C. (2005). *Geminiviridae*. Pages 301-326 in: Virus Taxonomy, VIIIth Report of the ICTV. C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball, eds. Elsevier/Academic Press, London.

Storey, H. H. (1936). Virus diseases of East African plants. VI. A progress report on studies of the disease of cassava. *East African Agricultural Journal* 2: 34–39. `

Storey, H.H. and Nichols, R.F.W. (1938). Studies on the mosaic of cassava. *Annals of Applied Biology* 25: 790-806.

Swanson, M.M. and Harrison, B.D. (1994). Properties, relationships and distribution of cassava mosaic geminiviruses. *Tropical Science* 34: 15-25.

Taylor, N.J. and Fauquet, C. (1997). Transfer of Rice and Cassava Gene Biotechnologies to Developing Countries. *Biotechnology International* 1: 239-246.

Tewe, O.O. and Lutaladio, N. (2004): The global cassava development strategy. International fund for agricultural development and food and agricultural organization, Rome, Itraly.

Thomas, J.E., Massalski, P.R. and Harrison, B.D. (1986). Production of monoclonal antibodies to African cassava mosaic virus and differences in their reactivities with other whitefly-transmitted geminiviruses. *Journal of General Virology* 67:2739-2748.

Thompson, R.J., Dasgupta, I., Fuchs, M., Iwanami, T., Karasev, A.V., Petrzik, K….and ICTV Report Consortium. (2017). ICTV Taxonomy Profile. *Journal of General Virology,*98:529–531.

Thottapilly, G., Thresh, J.M., Calvert, L.A and Winter, S. (2003). *Cassava*. P 107-165 In *Virus*and virus-like diseases of major crops in developing countries. G. leobenstein and G Thottapilly (eds) kluwer academic published., Dordrecht, The Nertherlands.

Thresh, J.M. Fishpool, L.D., Otim-Nape, G.W and Fargette, D. (1994). African cassava mosaic virus disease: An under-estimated and unsolved problem. *Tropical Science.* 34: 3-14.

Thresh, J.M., Otim-Nape, G.W., Legg, J.P. and Fargette, D. (1997). African cassava mosaic virus disease the magnitude of the problem. *African Journal of Root and Tuber Crops.* 2: 13-19.

Thurston, H.D. (1998).Tropical Plant Diseases. St. Paul: APS press.

Tivana, L.D. (2012). *A study of heap fermentation and protein enrichment in cassava*. University of Zimbabwe Institutional Repository. Pp. 1-6.

Wasswa, P., Alicai, T. and Mukasa, S.B.(2011). Optimisation of in vitro techniques for Cassava brown streak virus elimination from infected cassava clones. *African Crop Science Journal* 18: 235 – 241.

Westby, A. (2008). Cassava Utilization, Storage and Small-Scale Processing in Nigeria. In R Hillock, J. Thresh and A. C. Bellotti, eds., *Cassava Biology, Production and Utilization*, CABI Publishing. 9-11.

Winter, S., Koebler, M. Stein, B., Pietruszca, A., Paape, M and Butgereitt, A. (2010). The analysis of cassava brown streak virus species causing brown streak disease in East Africa. *Journal of General Virology*. 91: 365-376.

Zhou, X., Robinson, D.J.and Harrison, B.D. (1997). Types of variation in DNA-A among isolates of East African cassava mosaic virus from Kenya, Malawi and Tanzania. *Journal of General Virology* 79: 2835-2840.

APPENDICES

**Appendix I: Information collected from farmers via questionnaires**

| Location | Farm size (ha) | Cropping pattern | Crop surrounding the plant | Age of plant (months) | Cassava variety | Origin of cutting | Chemical treatment |
|---|---|---|---|---|---|---|---|
| Jar kasa | 2.19 | Mixed | cowpea, groundnut | 8 | Local | Previous harvest | Nil |
| Dutsawa | 2.72 | Mixed | Potato, cowpea | 7 | Local | Previous harvest | Nil |
| Kuya | 0.22 | Sole | Onion, tomato | 8 | Local | Previous harvest | Nil |
| Makiyaya | 0.92 | Mixed | Maize, potato | 9 | Local | Previous harvest | Nil |
| Mahuta | 1.92 | Mixed | G/nut, sorghum | 9 | Local | Previous harvest | Nil |
| Wagini | 0.46 | Sole | Potato, kenaf | 6 | White | Previous harvest | Nil |
| Yalwa | 0.13 | Sole | Maize, cowpea | 6 | Local | Previous harvest | Nil |
| Ganjirawa | 0.66 | Mixed | Maize and cowpea | 3 | Local | Previous harvest | Nil |
| G/ makera | 0.35 | Sole | Maize, sorghum | 6 | Local | Previous harvest | Nil |
| Michika | 0.21 | Mixed | Sorghum, cowpea | 7 | Local | Previous harvest | Nil |
| Bare-bari | 0.02 | Mixed | Sorghum, g/nut | 7 | Local | Previous harvest | Nil |
| Michika gari | 0.09 | Mixed | Maize, cowpea | 9 | Local | Previous harvest | Nil |
| Nassarawa | 0.08 | Mixed | Onion, tomato | 8 | Local | Previous harvest | Nil |
| Koye | 0.66 | Sole | Maize, cowpea | 8 | Local | previous harvest | Nil |
| Wudil | 0.04 | Mixed | Tomato, pepper | 7 | Local | Previous harvest | Nil |
| Kayu | 0.77 | Sole | Maize, millet | 7 | Local | Previous harvest | Nil |
| Madigawa | 1.03 | Mixed | Maize | 6 | Local | Previous harvest | Nil |

**Appendix II:** Coordinates, Elevation, farm size and name of villages surveyed

| State | Local government | Village | Farm size | Coordinates | Elevation |
|---|---|---|---|---|---|
| Katsina | Bakori | Gidan makera | 0.353ha | N 11$^{O}$39.172<br>E007$^{O}$30.133mins | 661metres |
| | | Ganjirawa | 0.6573ha | N11$^{O}$39.661mins<br>E007$^{O}$28.907mins | 647metres |
| | | Yalwa | 0.1266ha | N11$^{o}$38.968mins<br>E007$^{O}$28.503secs | 646metres |
| | Batsari | Wagini | 0.4584ha | N12$^{o}$41.410mins<br>E007$^{O}$11.119secs | 492metres |
| | | Makiyaya | 0.9162ha | N12$^{o}$43.644mins<br>E007$^{O}$10.916 | 505metres |
| | | Mahuta | 1.9219ha | N12$^{O}$06.682mins<br>E008$^{o}$31.989sec | 473metres |
| | Dutsi | Kuya | 0.2151ha | N12$^{O}$50.313secs<br>E008$^{O}$0.765mins | 499metres |
| | | Dutsawa | 2.715ha | N12$^{O}$50.306<br>E008$^{O}$07.844 | 496metres |
| | | Jar kasa | 2.193ha | N12$^{0}$49.959<br>E008$^{O}$04.975 | 522metres |
| Kano | Wudil | Kayu | 0.7654ha | N11$^{O}$53.546<br>E007$^{O}$56.347 | 585metres |
| | | Tsibiri I | 0.0546ha | N11$^{O}$49.692secs<br>E008$^{O}$51.298 | 408metres |
| | | Tsibiri II | 0.0385ha | N11$^{O}$49.559<br>E008$^{O}$51.325 | 406metres |
| | Ungogo | Michika | 0.345ha | N12$^{O}$05.899<br>E008$^{O}$30.845 | 481metres |
| | | Michika gari | 0.0908 | N12$^{O}$06.779<br>E008$^{O}$32.424 | 468metres |
| | | Bare-bari | 0.234ha | N12$^{O}$06.027<br>E008$^{O}$31.106 | 480metres |
| | Gwarzo | Madigawa | 1.0345ha | N11$^{O}$53.385<br>E007$^{O}$56.255 | 591metres |
| | | Koye | 0.657ha | N11$^{O}$53.362<br>E007$^{O}$56.221 | 590metres |
| | | Nassarawa | 0.0832 | N11$^{O}$49.534<br>E008$^{O}$51.305 | 407metres |

**Appendix III: Buffers prepared for use in the TAS and DAS-ELISA procedure**

| Buffer | Reagents | Dilution of reagents |
|---|---|---|
| Coating buffer | 1.59 g sodium carbonate ($Na_2C0_3$)<br>2.93 g sodium bicarbonate ($NaHC0_3$)<br>0.20 g sodium azide ($NaN_3$) | Dissolved in 900 ml distilled water and filled to one litre.<br>Then adjusted to pH 9.6 ( refrigerated) |
| Phosphate buffered saline | 8.0 g sodium chloride (NaCl)<br>0.2 g monobasic potassium phosphate ($KH_2P0_4$) | Dissolved in 900 ml distilled water and filled to one litre<br>Then adjusted pH 7.4 with HCl |
| PBST -Tween (PBST) | PBS + 0.5 ml Tween 20 per litre | 0.5 ml Tween 20 was added to 99.5 ml PBS<br>Then adjusted to pH 7.4 with HCl |
| Sample extraction buffer | PBST + 2% PVP (serva PVP- 15 polyvinyl pyrrolidone) | 20 ml of PVP was added to 980 ml of PBST<br>Then adjusted to pH 7.4 with HCl |
| Conjugate buffer | PBST + 2% PVP + 2% egg albumin (sigma A- 5253) | 2.0 g of egg albumin (sigma A-5253) was added to 998.0 ml of sample extraction buffer. Stored at room temperature. |
| Substrate buffer | 97.0 ml diethanolamine<br>0.2 g sodium azide ($NaN_3$) | 600 ml distilled water was dissolved inand adjusted to pH 9.8 with HCl and filled to one litre |